Epigenetic control of oncogene signaling

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Memorial Sloan Kettering Cancer Center
May 13, 2024

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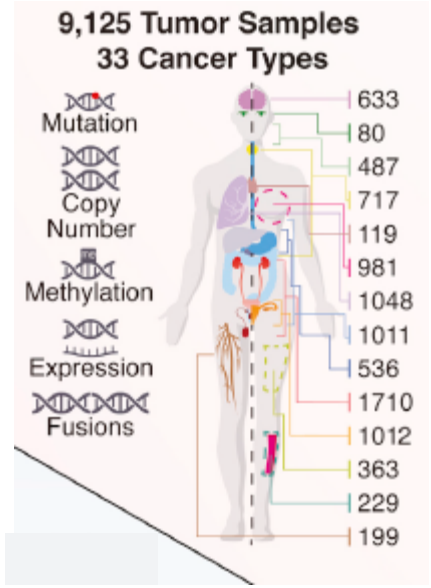
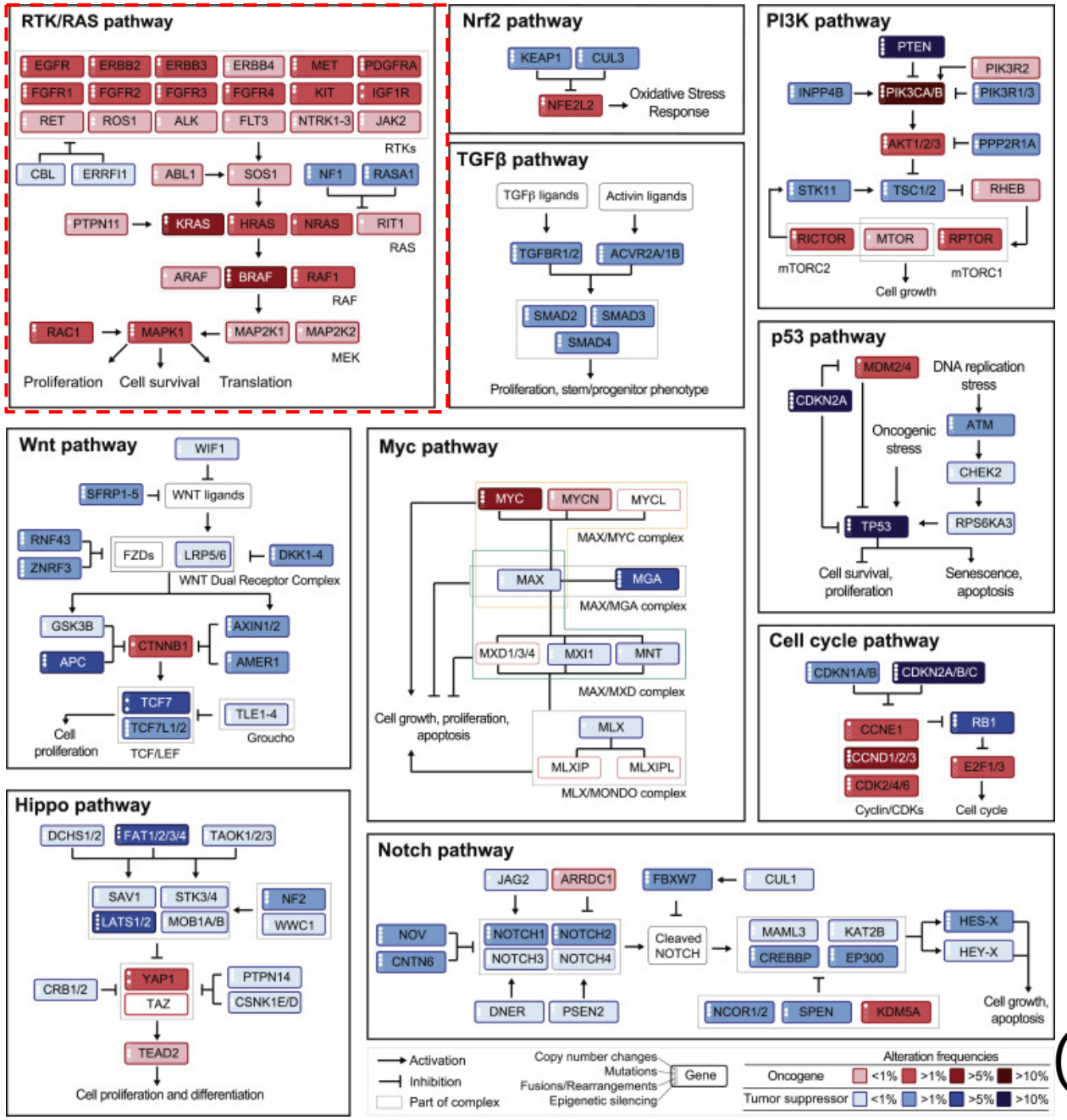
Oncogenic signaling pathways in cancer



89% ≥1 driver alteration in the pathways
57% ≥1 druggable targets



Sanchez-Vega, F., et al., Cell 2018

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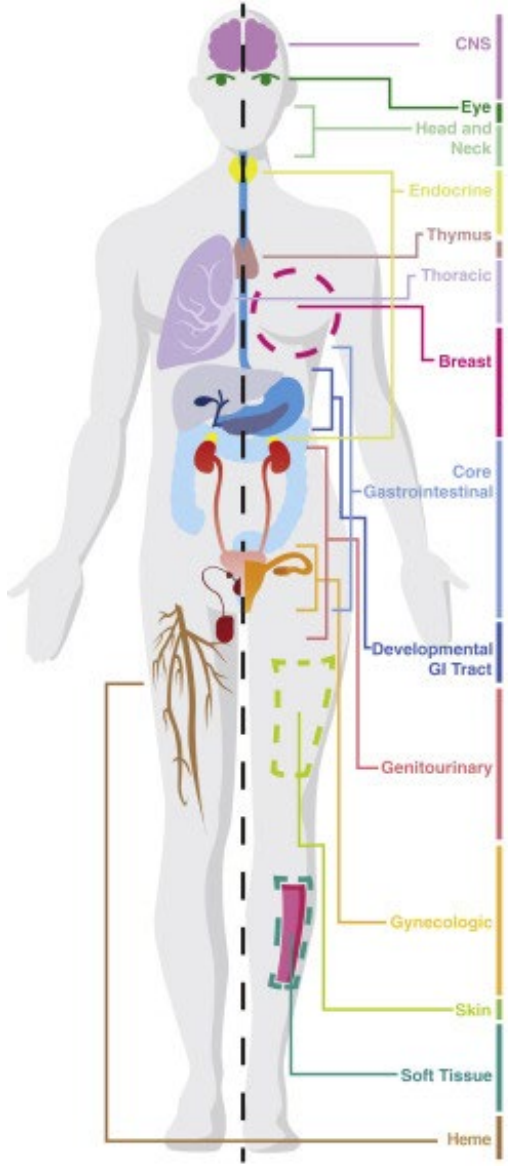
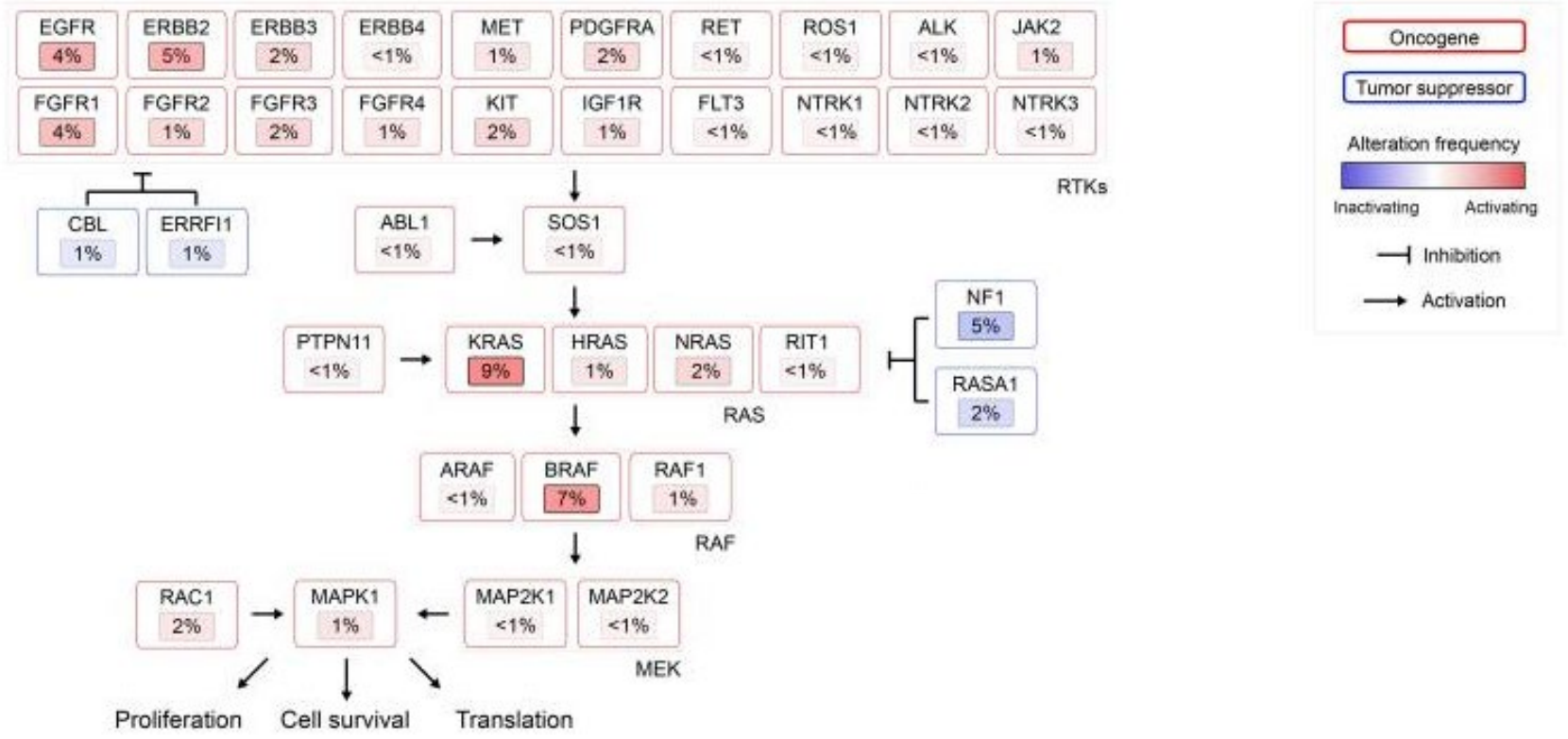
RTK signaling in cancer



Alteration frequencies	RTK/RAS	Cell cycle	PI3K	p53	Notch	Wnt	Myc	Hippo	TGFβ	Nrf2	CIN	FGA	TMB
GBM	77	86	57	48	18	8	6	10	2		119	0.37	4.3
LGG IDHwt	82	64	47	29	27	5	1	5			68	0.25	4.3
LGG IDHmut-codel	9	45	22	5	26	66	3	99	50		34	0.21	0.8
LGG IDHmut	19	28	15	92	24	8	10	92	21	1	60	0.17	0.8
UVM	6	6	4	2	10	1	2	10		1	65	0.28	0.4
HNSC HPV+	26	32	60	11	25	8	4	8	11	1	83	0.32	3.3
HNSC HPV−	45	86	39	82	36	16	20	42	13	13	108	0.43	4.6
THCA	84	14	4	1	4	13	2	1	2		16	0.03	0.4
ACC	22	30	16	28	11	41	7	5	1		146	0.78	1.8
PCPG	32	15	6	6	11	10	1	4	1		81	0.33	0.3
THYM	14	9	4	7	5	7	1	7	3	2	26	0.09	0.6
LUAD	74	56	38	61	21	19	23	23	10	15	118	0.48	8.2
MESO	9	54	13	21	9	6	7	40	2		98	0.41	0.8
LUSC	54	79	68	86	31	18	12	28	11	25	158	0.61	7.7
BRCA LumA	28	31	62	25	14	15	12	5	4	1	101	0.34	2.0
BRCA LumB	44	48	48	49	25	31	26	15	10	2	211	0.60	2.0
BRCA Her2-enriched	82	40	60	78	18	17	29	10	8	1	230	0.53	4.3
BRCA Basal	46	51	53	91	38	11	39	14	8	4	246	0.67	2.7
BRCA Normal	36	36	33	31	3	6	19	3			53	0.16	1.5
STES Squamous	50	89	53	96	38	13	22	21	13	23	189	0.59	3.1
STES CIN	63	74	33	76	21	26	21	16	23	2	222	0.58	3.4
STES EBV	50	100	80	13	83	67	7	10	17		52	0.22	4.1
STES GS	31	39	18	24	31	20	12	4	20	2	66	0.10	2.1
STES MSI-POLE	71	64	64	49	79	70	19	54	57	2	85	0.19	37.1
CRC MSI-POLE	99	74	68	49	74	95	52	64	55	1	45	0.09	56.9
CRC GS	88	45	53	19	29	90	21	10	38	5	55	0.23	2.9
CRC CIN	66	36	32	84	23	91	17	8	22	1	115	0.54	2.9
LIHC	22	69	25	37	26	43	19	12	7	7	121	0.45	2.9
CHOL	56	53	17	19	8	17	19	17	3	6	100	0.58	1.8
PAAD	78	70	19	69	14	12	14	7	41		62	0.26	3.5
KIRC	14	14	17	6	8	7	5	5	3	3	49	0.25	1.6
KIRP	17	12	8	4	12	9	6	11	1	6	49	0.35	2.2
KICH	5	23	15	32	3	3	2	3	5		77	0.80	0.9
BLCA	64	81	46	62	42	20	18	26	9	9	150	0.50	6.8
PRAD	15	28	32	21	13	35	11	5	6	1	92	0.16	1.3
TGCT sem	63	8	11	6	6		2				70	0.54	0.4
TGCT non−sem	20	7	5	5	16	2		10	2		99	0.67	0.4
OV	58	48	49	96	28	10	40	21	5	5	316	0.79	2.4
UCEC CN high	61	43	86	90	32	18	31	13	5	5	296	0.67	1.9
UCEC CN low	37	9	95	10	14	54	10	7	1	5	42	0.15	2.1
UCEC MSI-POLE	71	31	98	42	64	70	30	55	31	19	30	0.08	71.2
UCS	61	70	79	91	54	18	27	16	4	4	247	0.71	3.5
CESC Adeno	63	21	56	19	30	14	16	14	21	5	95	0.36	3.6
CESC Squamous	32	19	59	12	35	12	5	33	11	10	101	0.44	5.2
SKCM	94	77	33	28	27	23	10	25	7	1	131	0.53	22.1
SARC DDLPS	43	83	20	85	17	15	7	9	7		450	0.36	1.1
SARC LMS	31	55	33	71	14	11	4	4	1	4	177	0.69	1.8
SARC MFS/UPS	48	74	32	68	34	20	8	21	6	4	328	0.66	3.0
SARC other	25	30	15	5	5	5		10			104	0.33	1.2
DLBC	24	76	8	19	70	70	14	35	14		90	0.29	3.5
LAML	49	17	3	9	18	11	2	3	1	1	28	0.05	1.1
	46	45	33	29	23	15	11	10	7	1			

Sanchez-Vega, F., et al., Cell 2018

RTK signaling in cancer



Sanchez-Vega, F., et al., Cell 2018

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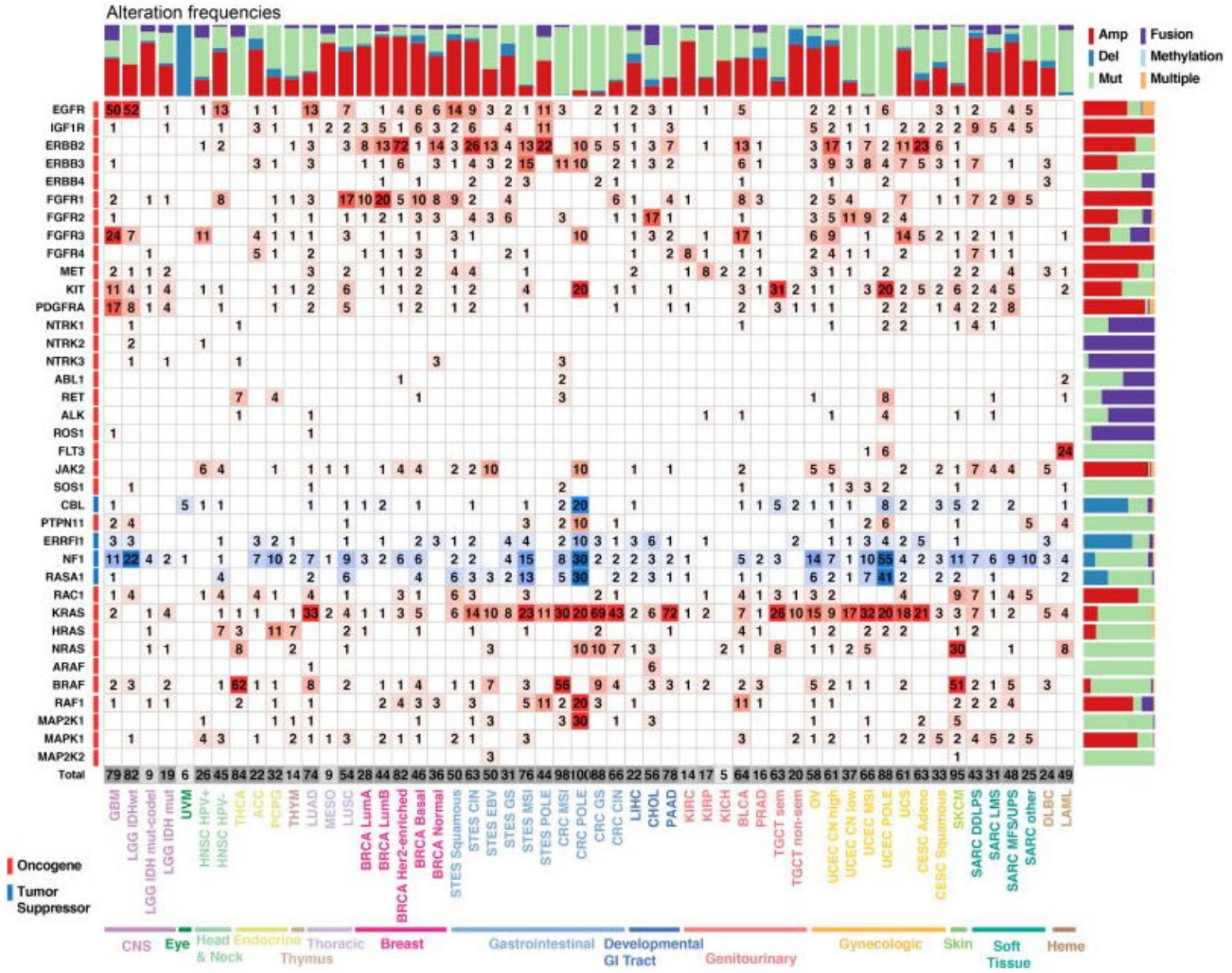
Genetic mechanisms of RTK activation in cancer



Genetic alterations

- Amplification
- Fusion
- Mutations
- Multiple

Sanchez-Vega F., et al., Cell 2018

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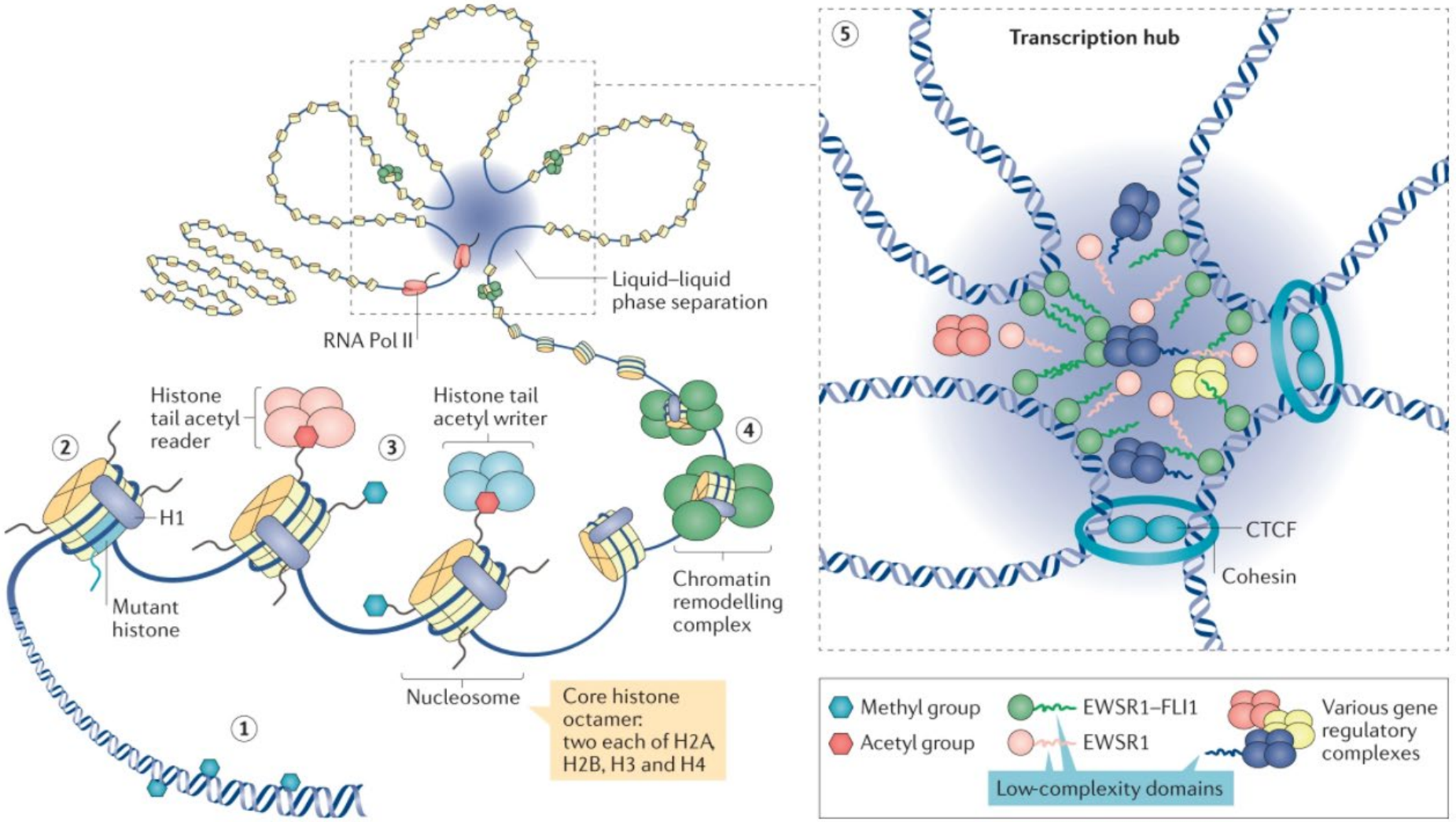
Epigenetic control of transcription



Nacev B et al., The epigenomics of sarcoma, NRC 2020

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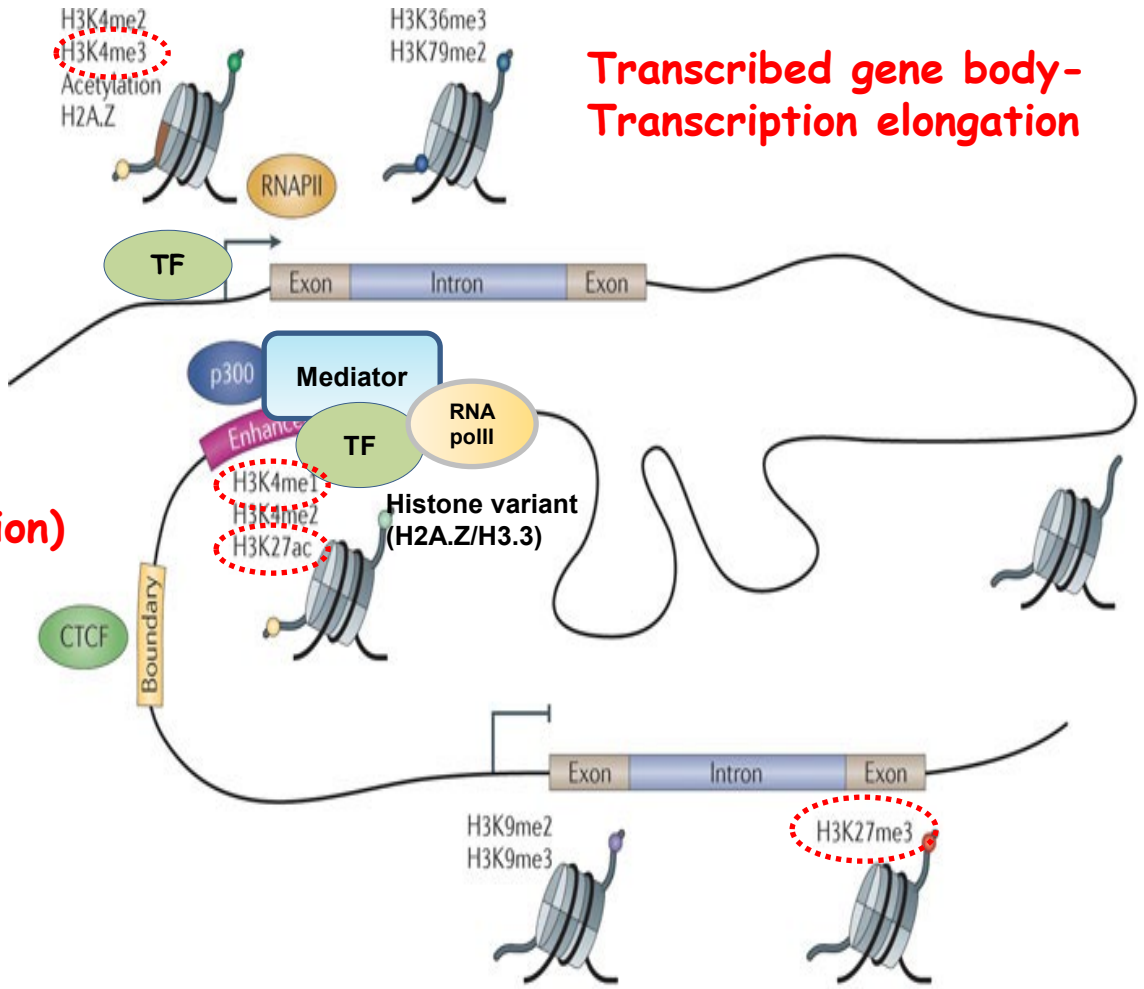
Features of transcription modules

(Active) Promoter (TSS)

(Active) Enhancers
(distal cis-regulatory region)

Silenced

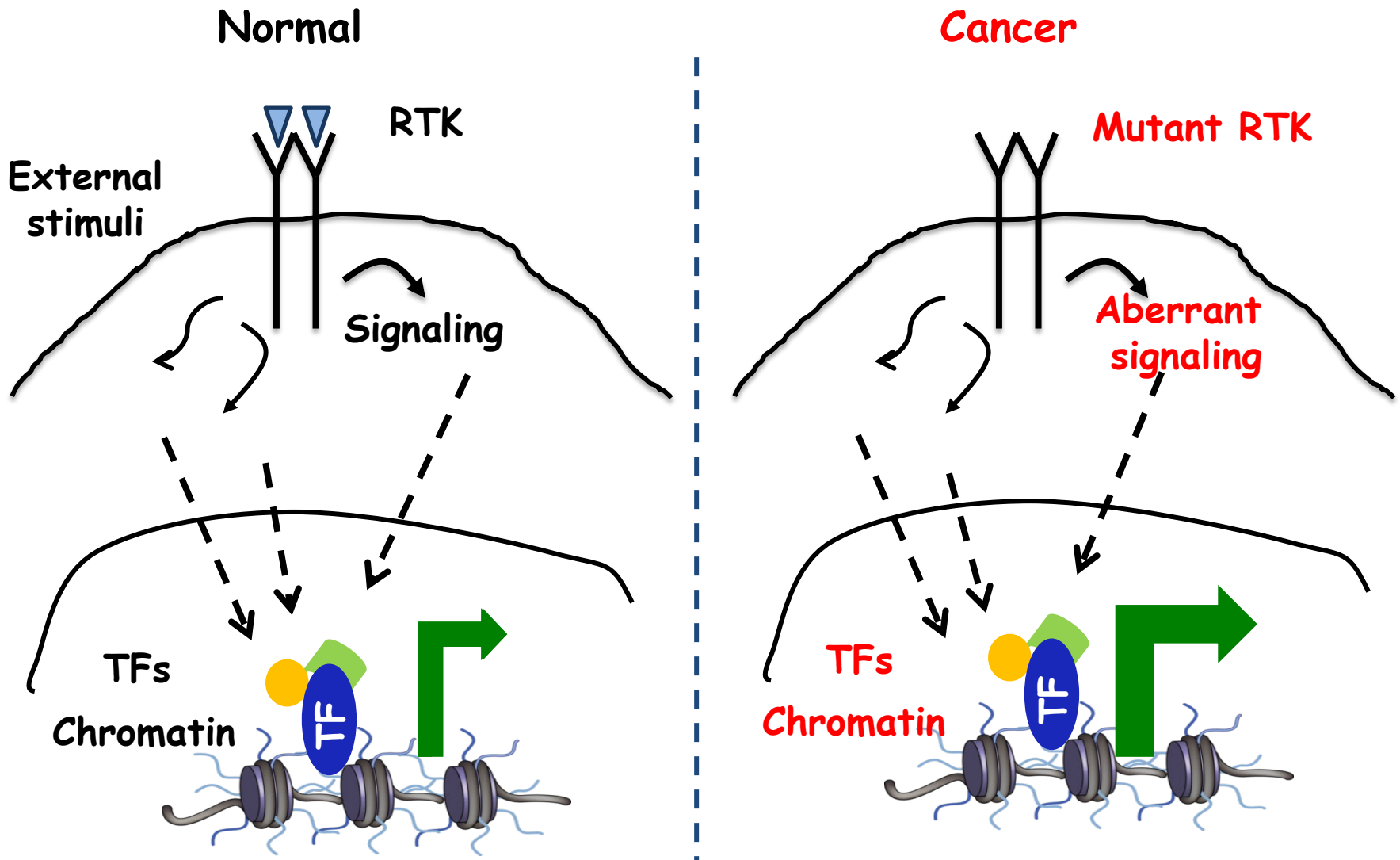


Nature Reviews | Genetics

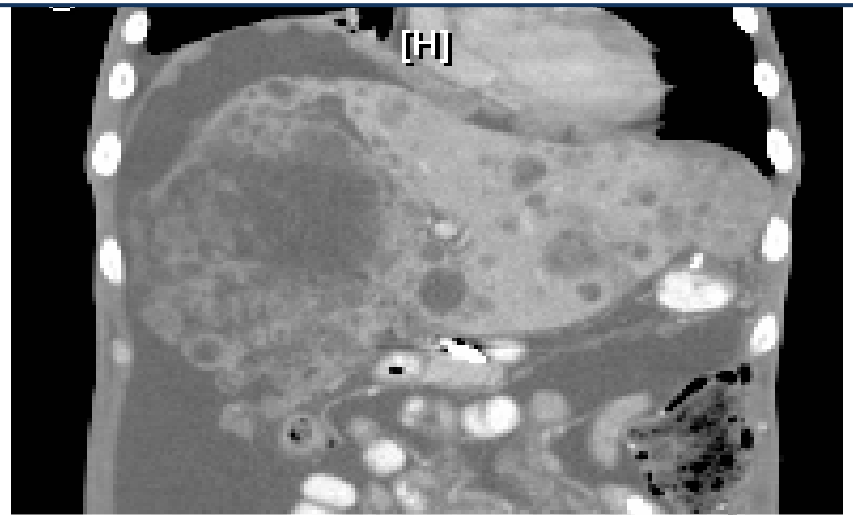
Transcribed gene body-
Transcription elongation

Adapted Zhou, Goren and Bernstein, NRG 2011;Calo and Wysocka, Mol Cell 2013

Genetic-epigenetic interaction in cellular context-dependent oncogenic transformation



Gastrointestinal stromal tumor (GIST)



Management:
- Surgery mainstay treatment
- Recurrence or metastatic disease - fatal
- Refractory to chemotherapy and radiation
- Imatinib is the first line therapy for past 20 years

- ~5,000 cases diagnosed per year in the US.

- One of the most common subtypes of soft tissue sarcomas, the most common mesenchymal neoplasm in the GI tract.

- Can arise anywhere from the entire GI tract; stomach is the most common primary site (2/3), then small bowel (1/4), esophagus/colon/rectum (the rest).

- Originates from Interstitial Cells of Cajal (ICCs).

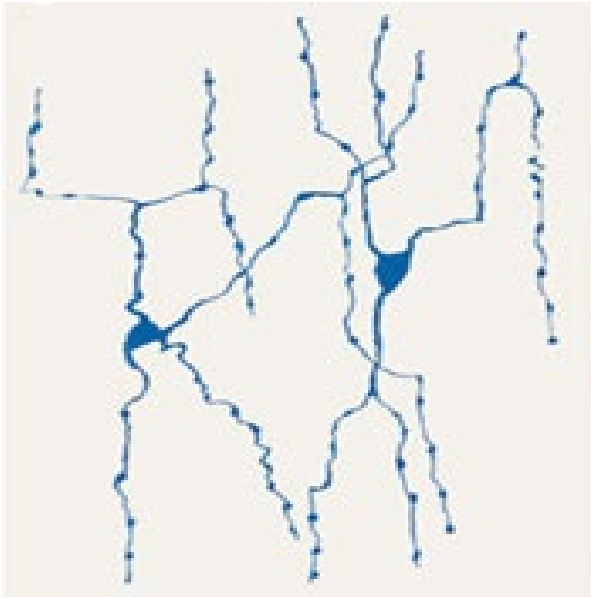
- The majority of GISTs are characterized by KIT or PDGFRA activating mutations, other mutations include BRAFV600E, NF1 loss, SDH-deficiency, etc.

- Peak incidence 50–65-year-old for sporadic GIST. Familial syndromes with early onset.

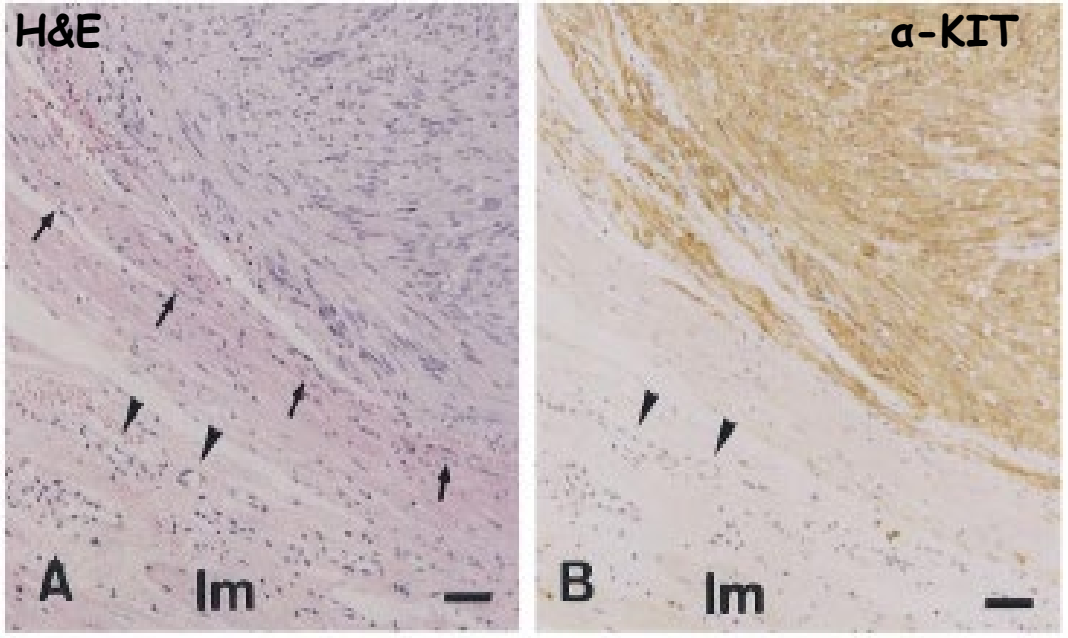
GIST originates from ICC naturally express KIT

- Originates from the Interstitial Cells of Cajal (ICCs) of the GI tract
- Characterized by KIT positive IHC and activating mutations in KIT or PDGFRA

Interstitial Cell of Cajal (ICC)- Pacemaker cells of the GI tract



GIST of stomach



Huizinga, J.D. et al., Nature, 1995; Hirota, S., et al., Science, 1998

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EFFICACY AND SAFETY OF IMATINIB MESYLATE IN ADVANCED GASTROINTESTINAL STROMAL TUMORS

GEORGE D. DEMETRI, M.D., MARGARET VON MEHREN, M.D., CHARLES D. BLANKE, M.D., ANNICK D. VAN DEN ABBEELE, M.D., BURTON EISENBERG, M.D., PETER J. ROBERTS, M.D., MICHAEL C. HEINRICH, M.D., DAVID A. TUVESON, M.D., PH.D., SAMUEL SINGER, M.D., MILOS JANICEK, M.D., PH.D., JONATHAN A. FLETCHER, M.D., STUART G. SILVERMAN, M.D., SANDRA L. SILBERMAN, M.D., PH.D., RENAUD CAPDEVILLE, M.D., BEATE KIESE, M.SC., BIN PENG, M.D., PH.D., SASA DIMITRIJEVIC, PH.D., BRIAN J. DRUKER, M.D., CHRISTOPHER CORLESS, M.D., CHRISTOPHER D.M. FLETCHER, M.D., AND HEIKKI JOENSUU, M.D.

N Engl J Med, Vol. 347, No. 7 · August 15, 2002

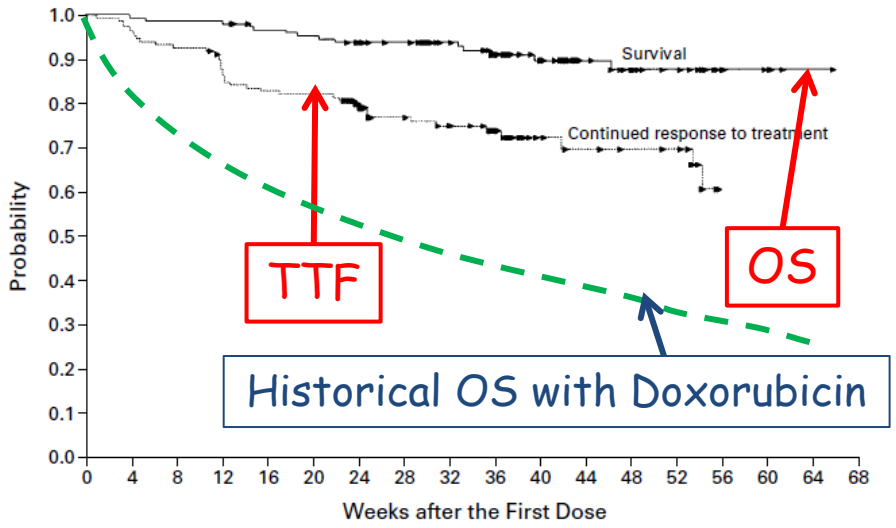
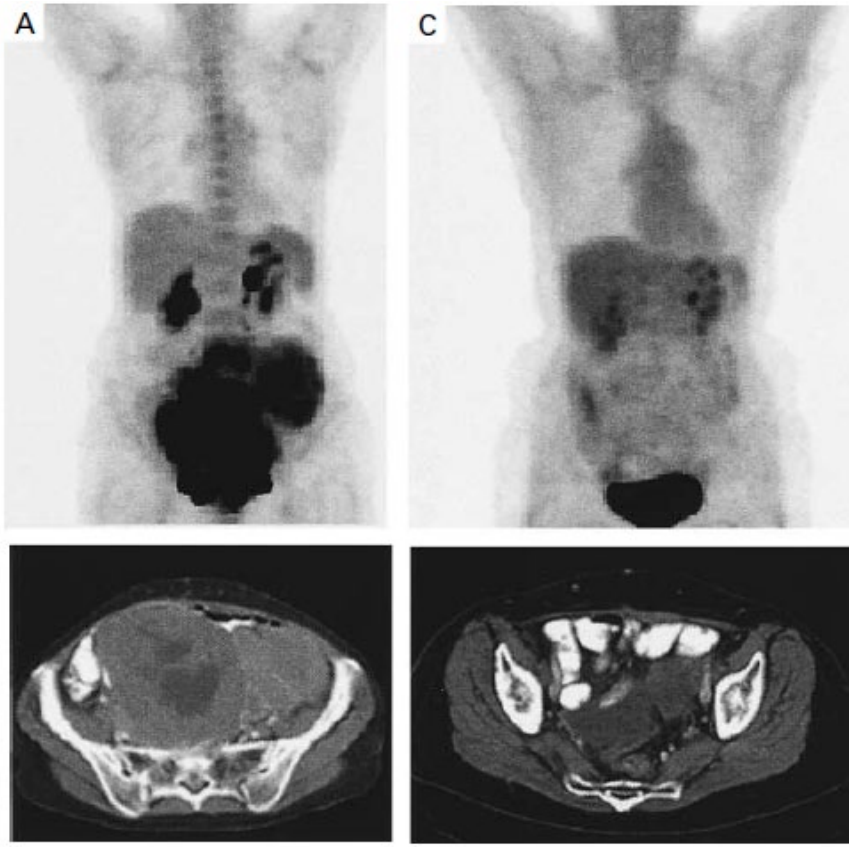


Figure 1. Kaplan–Meier Estimates of Overall Survival and Time to Treatment Failure for All Patients. Each arrowhead represents the point at which a patient's data were censored.



EORTC-62005
Phase III Trial (n = 377)[69]

SWOGS0033/CALGB150105
Phase III Trial (n = 428)[70]

Imatinib (Gleevec)-FDA approved as 1st line therapy for GIST 2002!
1st targeted therapy in solid tumor.

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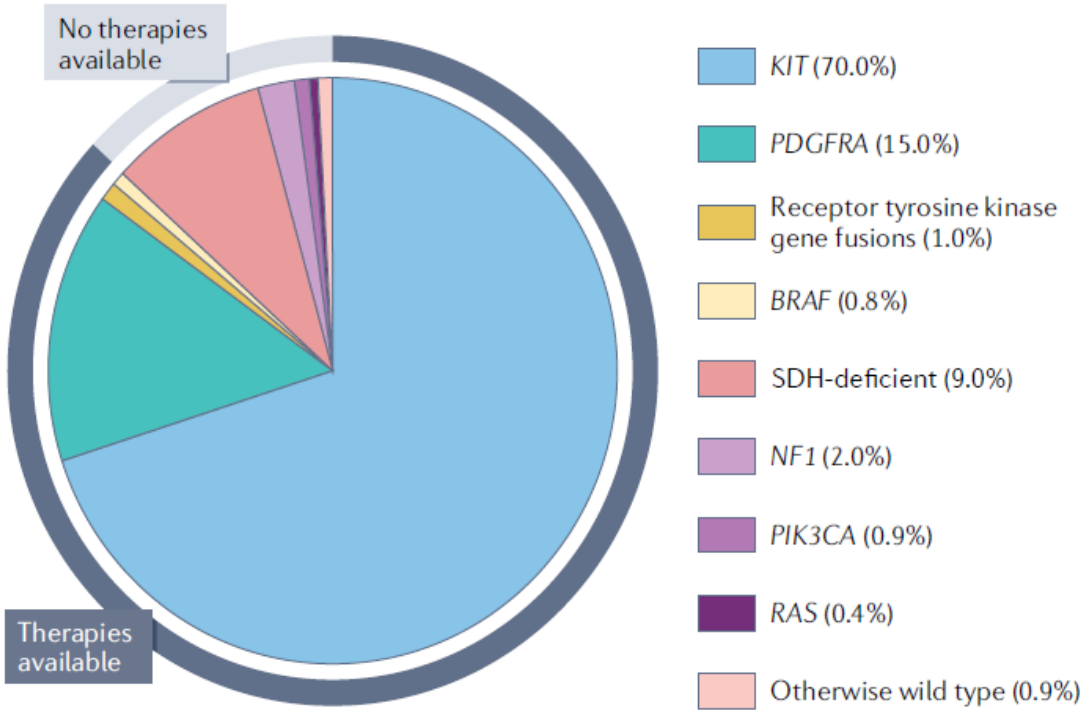
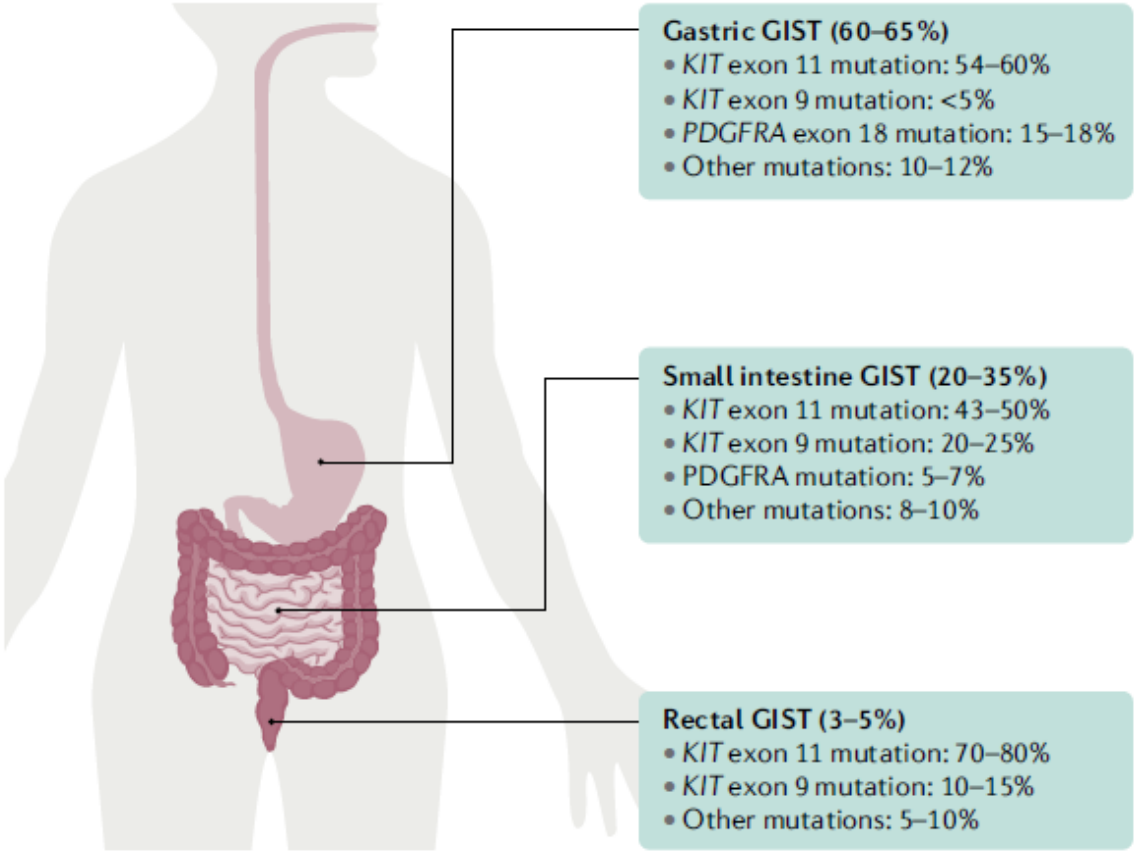
Molecular characterization of GIST



Klug LR et al., Nat Rev Clin Oncol, 2022



Blay JY et al., Nat Rev Dis Primers, 2021

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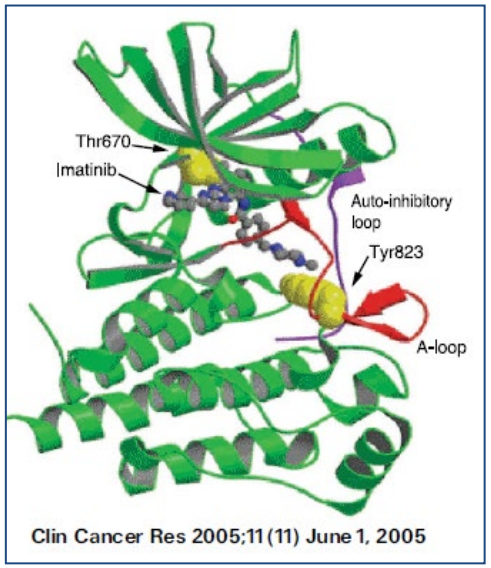
Clinical challenges- heterogeneous imatinib resistance mechanisms

14% - Primary resistance; 50% - Develop imatinib resistance within 2 years



Clin Cancer Res 2005;11(11) June 1, 2005

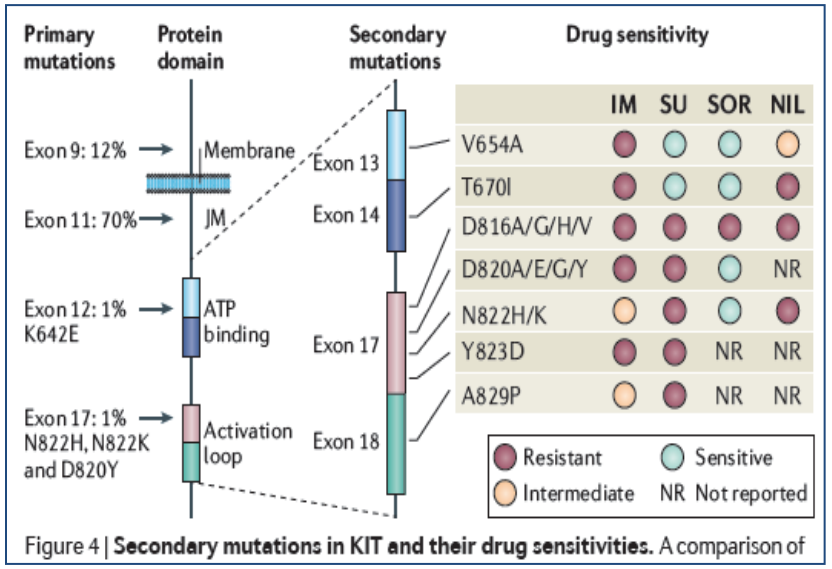
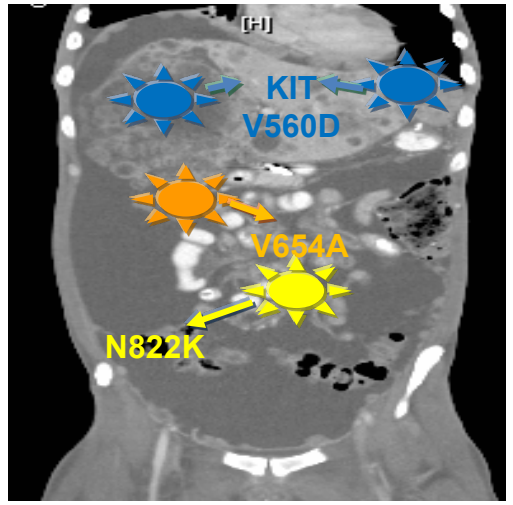


Figure 4 | **Secondary mutations in KIT and their drug sensitivities.** A comparison of

Polyclonal Resistance



Resistance Mechanisms:

1. Various secondary mutations (50-65%)
2. Genomic Amplification of RTKs
3. Activation alternative signaling pathways
4. Kit-low, imatinib-resistant GIST stem/progenitors
5. Tumor adaptation and persistence of disease
6. Tumor heterogeneity and polyclonal resistance
7. Others…

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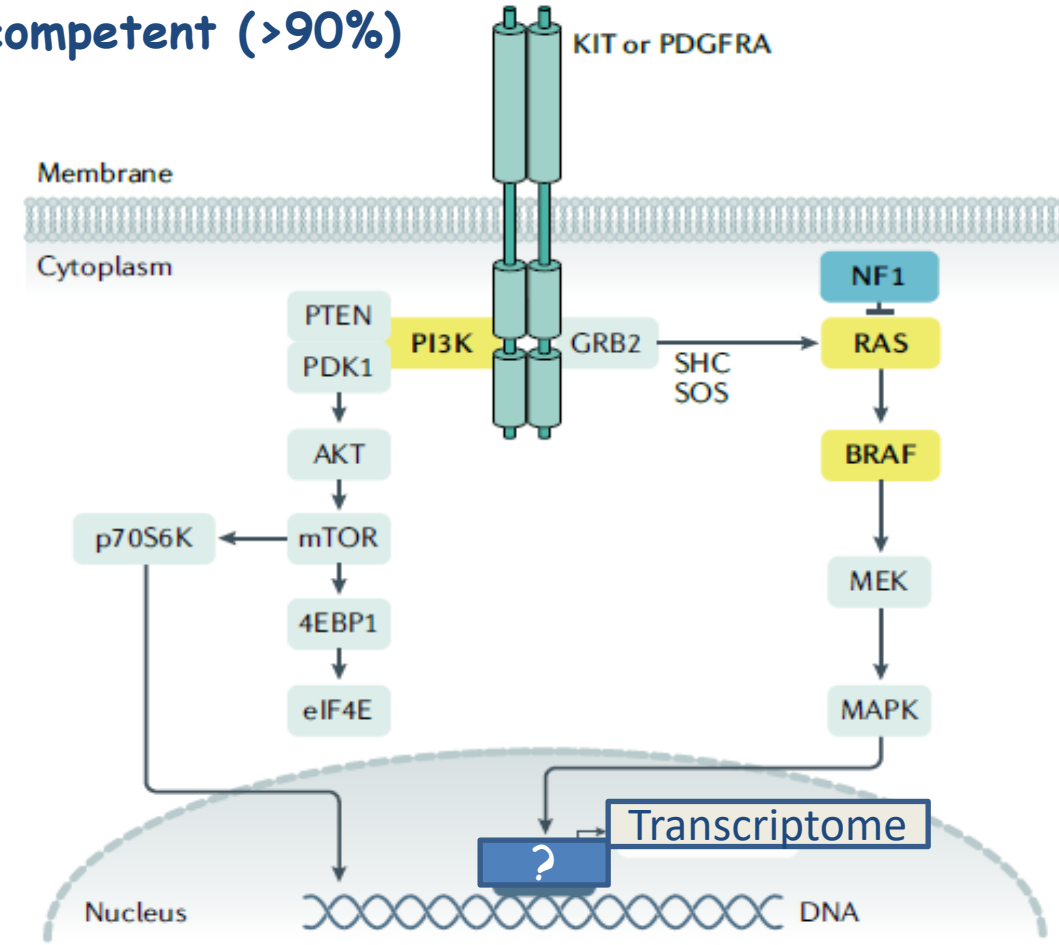
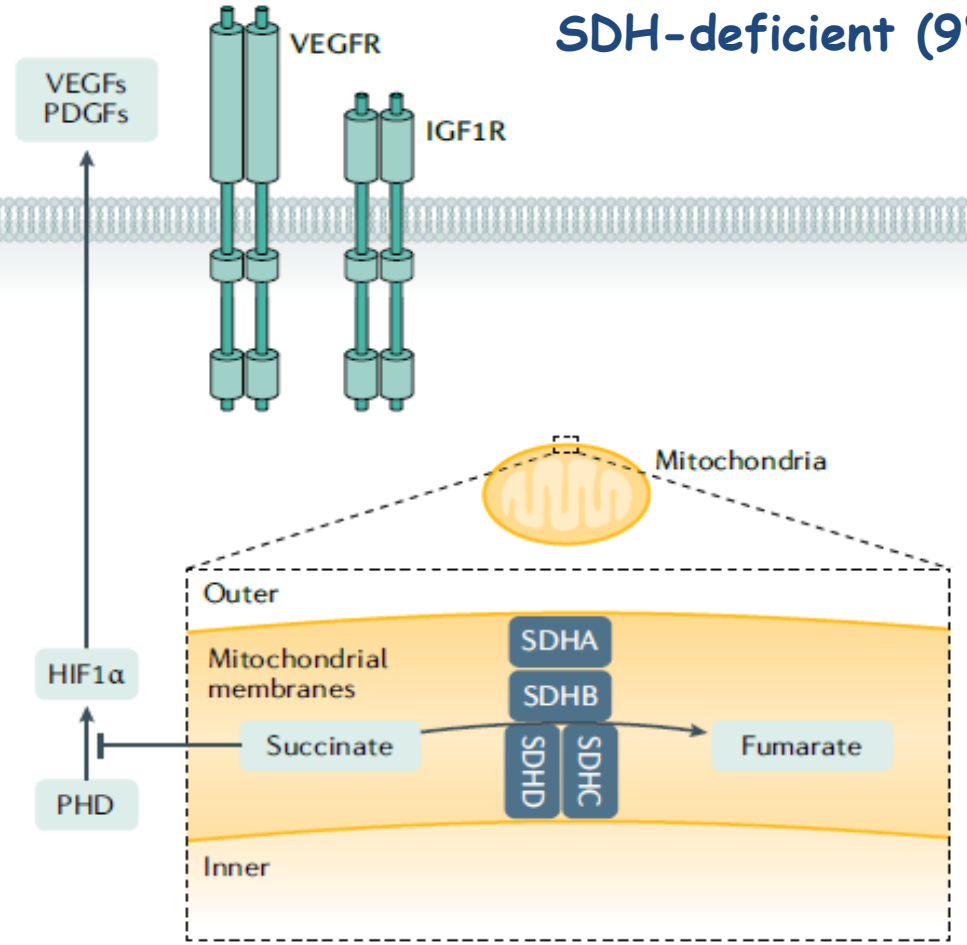
Molecular pathogenesis of GIST

SDH-competent (>90%)



SDH-deficient (9%)



Corless, et al, Nat Rev Cancer, 2011; Blay JY et al., Nat Re Dis Primers, 2021

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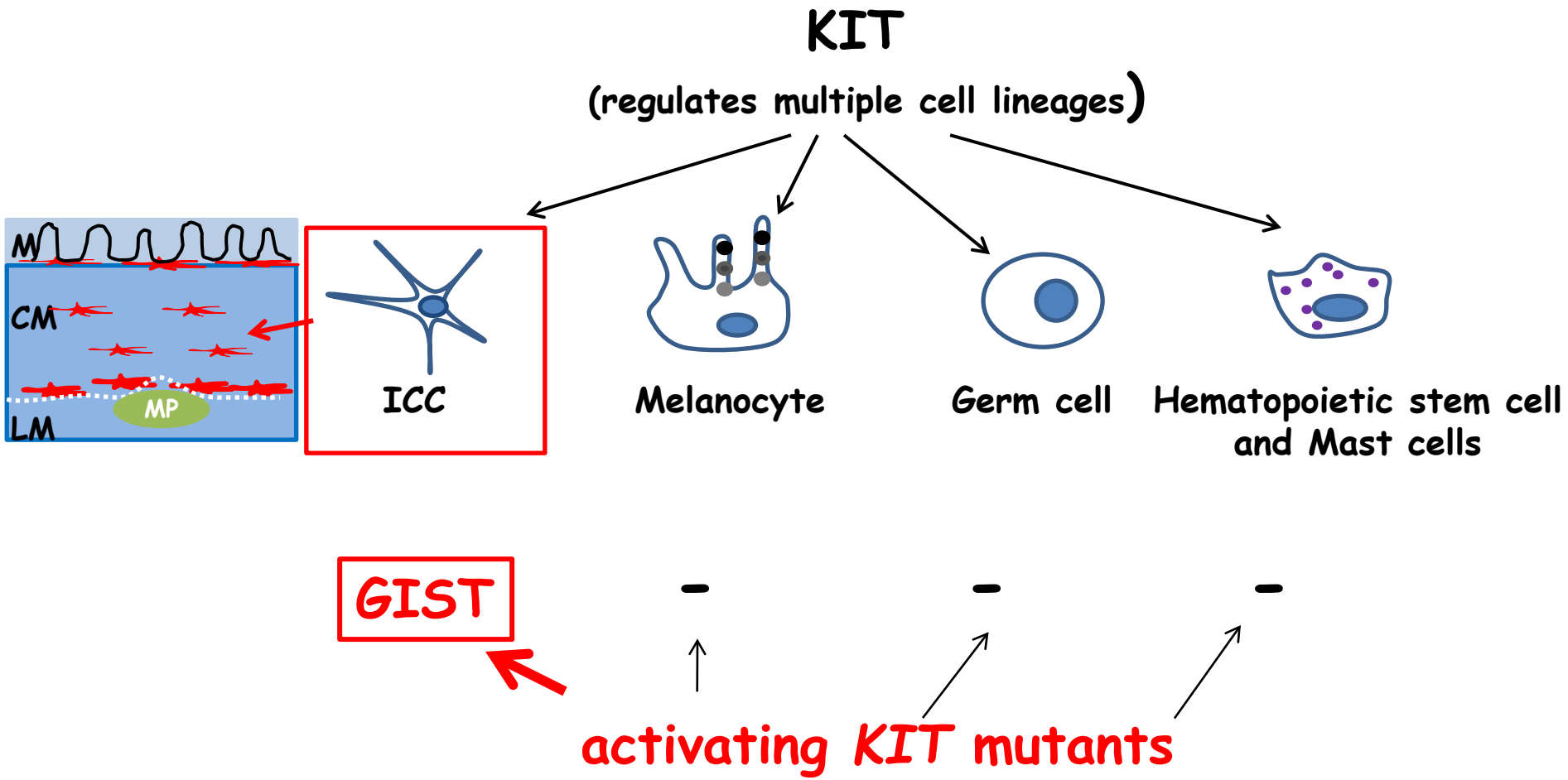
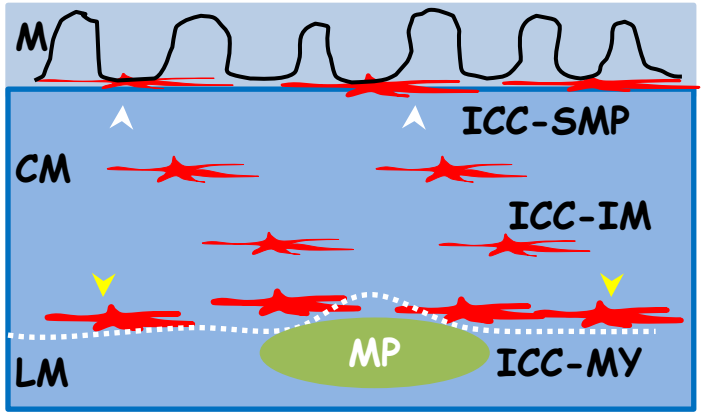
Familial gastrointestinal stromal tumor (GIST)



Familial GIST mouse models (Kit V558del, K641E, D818Y)

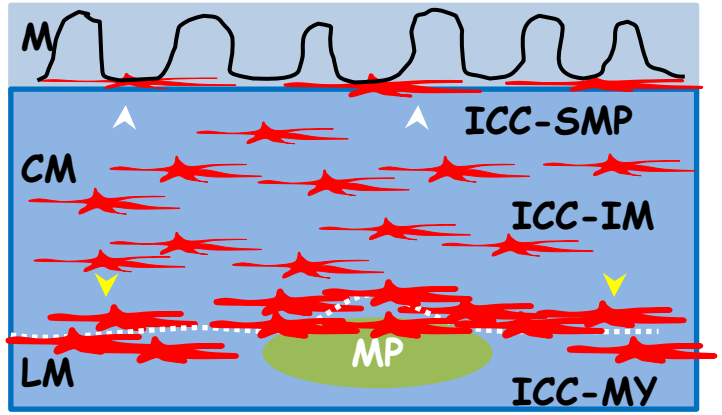
Control *Kit*$^{+/+}$

Large Intestine



$Kit^{\Delta 558/+}$

Large Intestine

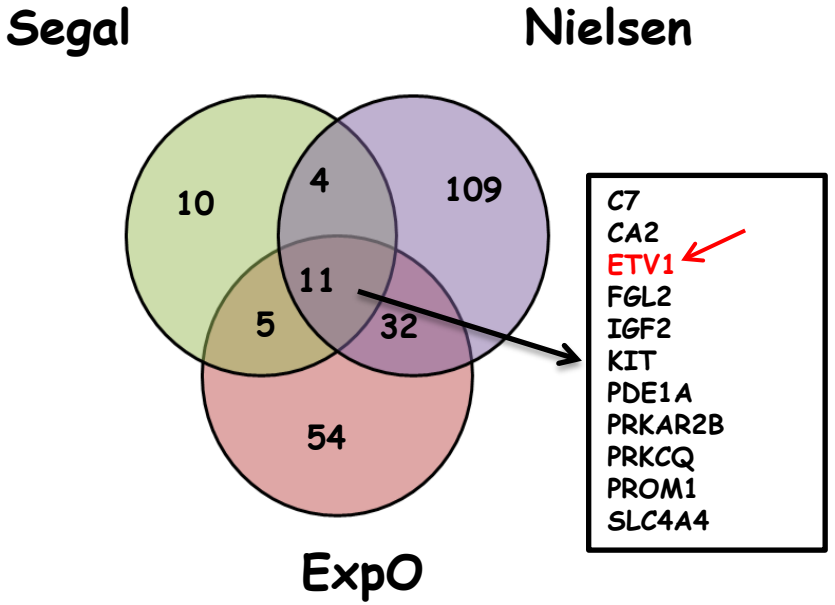


ICC-SMP

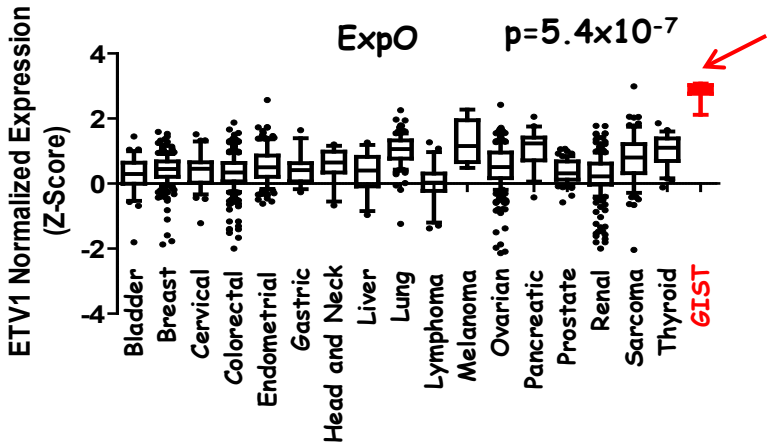
ICC-IM
ICC-MY

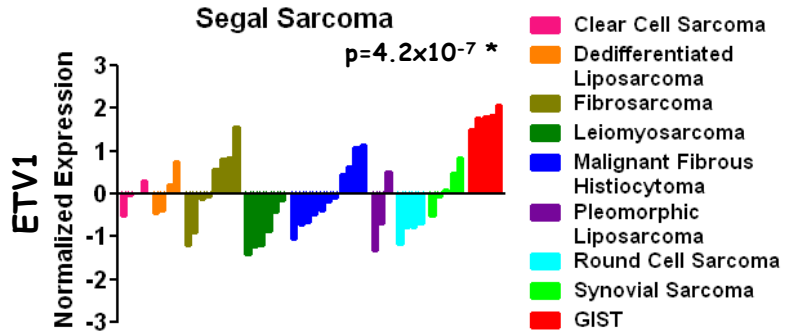
Sommer G et al, PNAS 2003; Rubin BP et al, Cancer Res 2005; Nakai N et al, J Pathol 2008; Kwon JG et al, Gastroenterology 2009

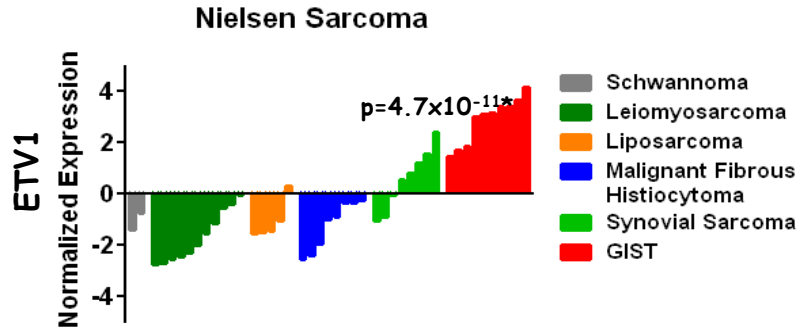
ETV1 is differentially highly expressed in GIST



q<0.05
Z difference >1.5







Segal NH et al., Am. J. Pathol 2003; Nielsen TO et al., Lancet 2002; ExpO (Expression Project for Oncology)

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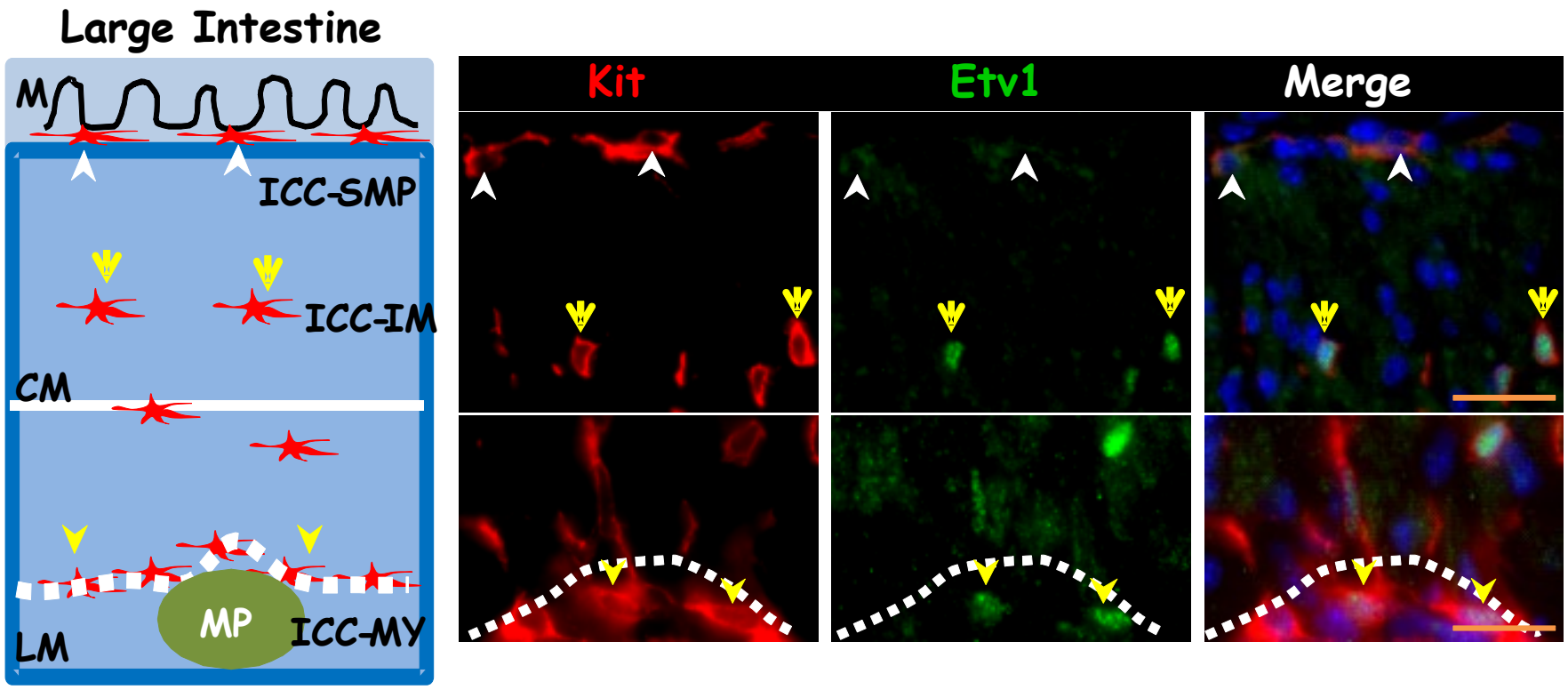
ETV1 is expressed in specific ICCs susceptible to oncogenesis



Chi P, Chen Y, et al, Nature, 2010

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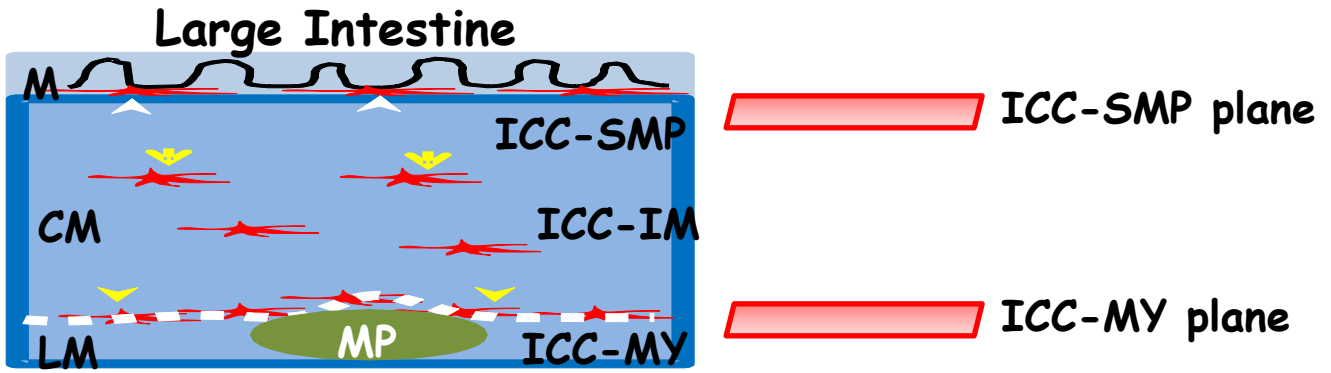
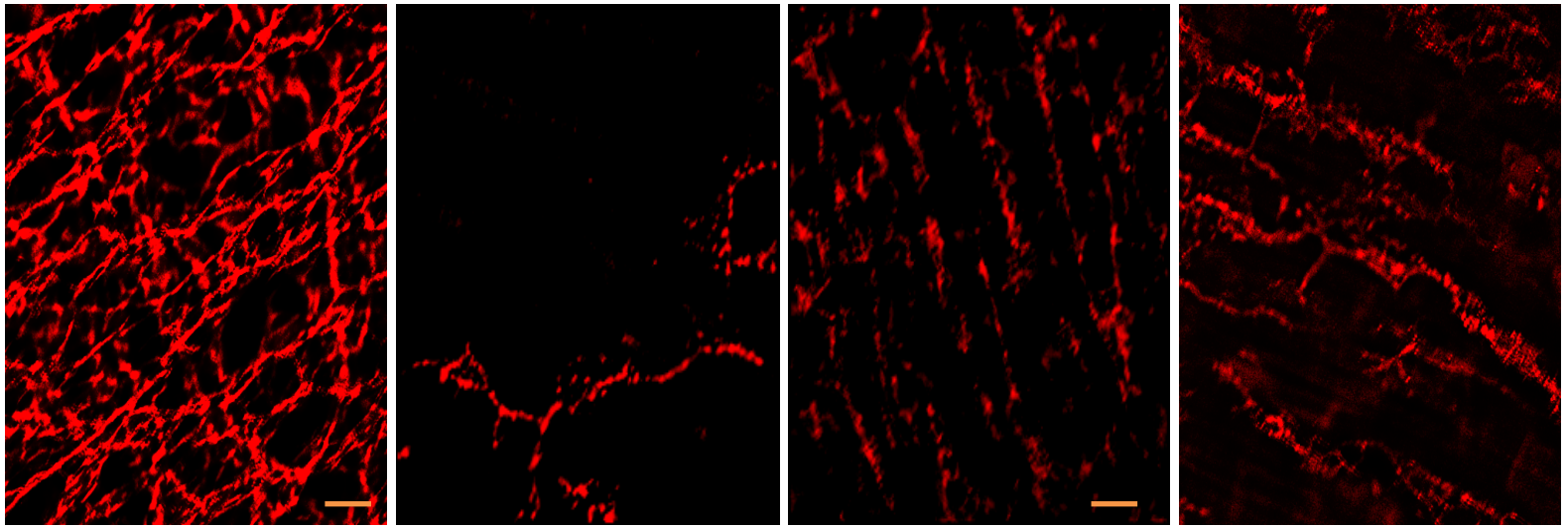
ETV1 is required for the development of ICC-IM /ICC-MY networks



Kit whole-mount (ICC-MY Plane) Kit whole-mount (ICC-SMP Plane)

Etv1 $^{+/+}$ *Etv1* $^{-/-}$ *Etv1*$^{+/+}$ *Etv1* $^{-/-}$



Chi P, Chen Y, et al, Nature, 2010

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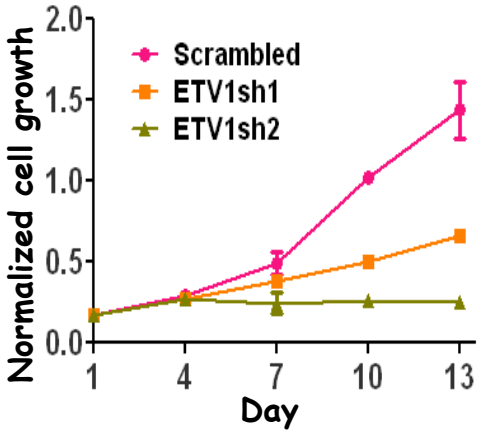
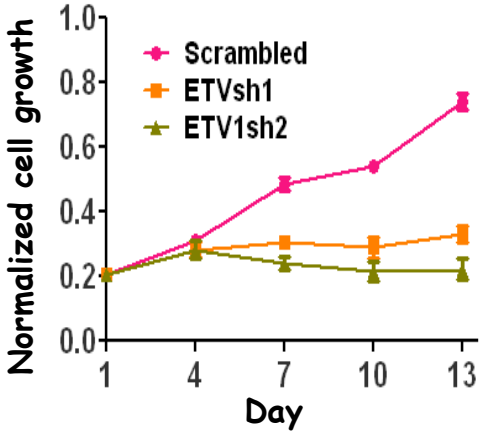
ETV1 is required for GIST growth and survival

GIST882 cell (imatinib sensitive)

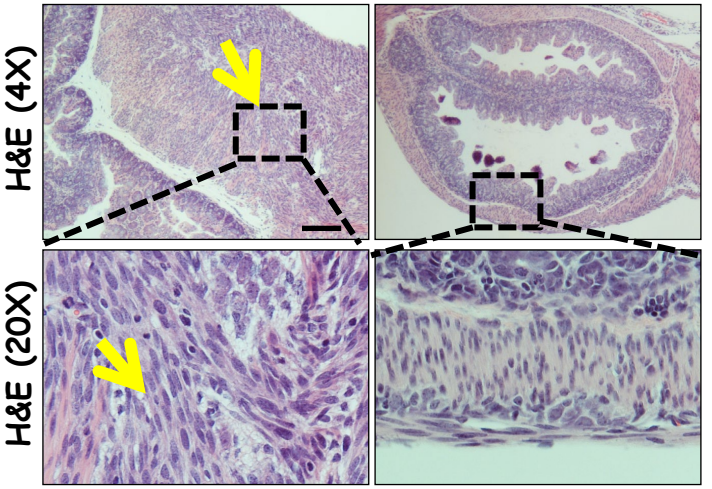


GIST48 cell (imatinib resistant)



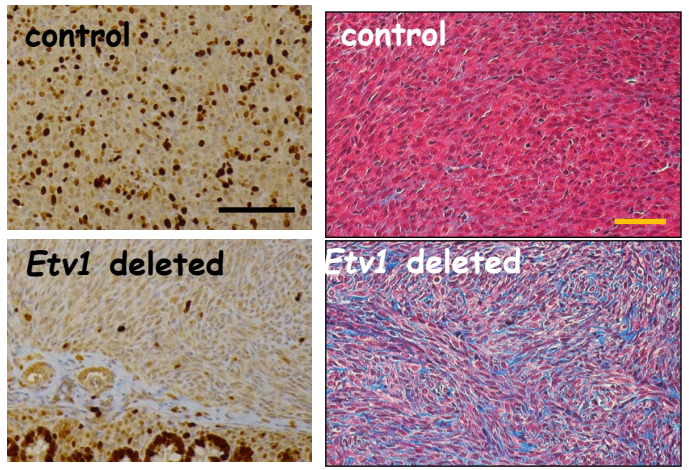
GEMM: $Kit^{\Delta 558/+}$

Cecum ($Etv1^{+/+};Kit^{V558\Delta/+}$) Cecum ($Etv1^{-/-};Kit^{V558\Delta/+}$)



GEMM: $Kit^{\Delta 558/+};Etv1^{flox/flox};Rosa26^{CreERT2/CreERT2}$)

Cecal tumor (Ki67 IHC) Cecal tumor (trichrome)



Chi P, Chen Y et al, Nature 2010; Ran L et al., Cancer Discovery, 2015

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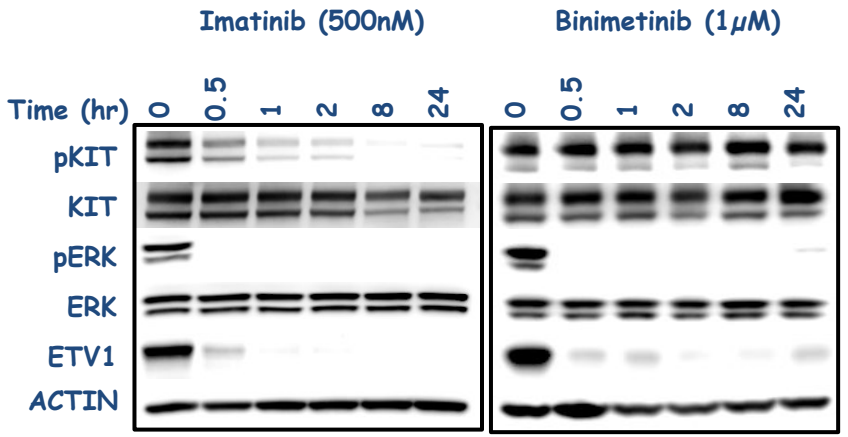
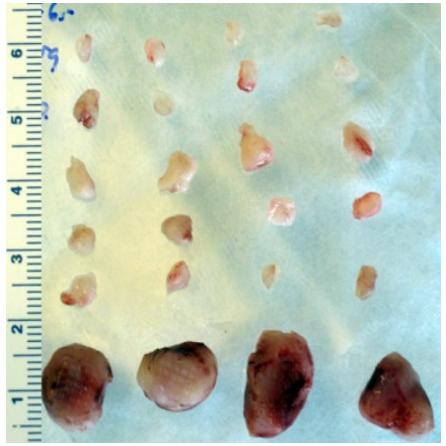
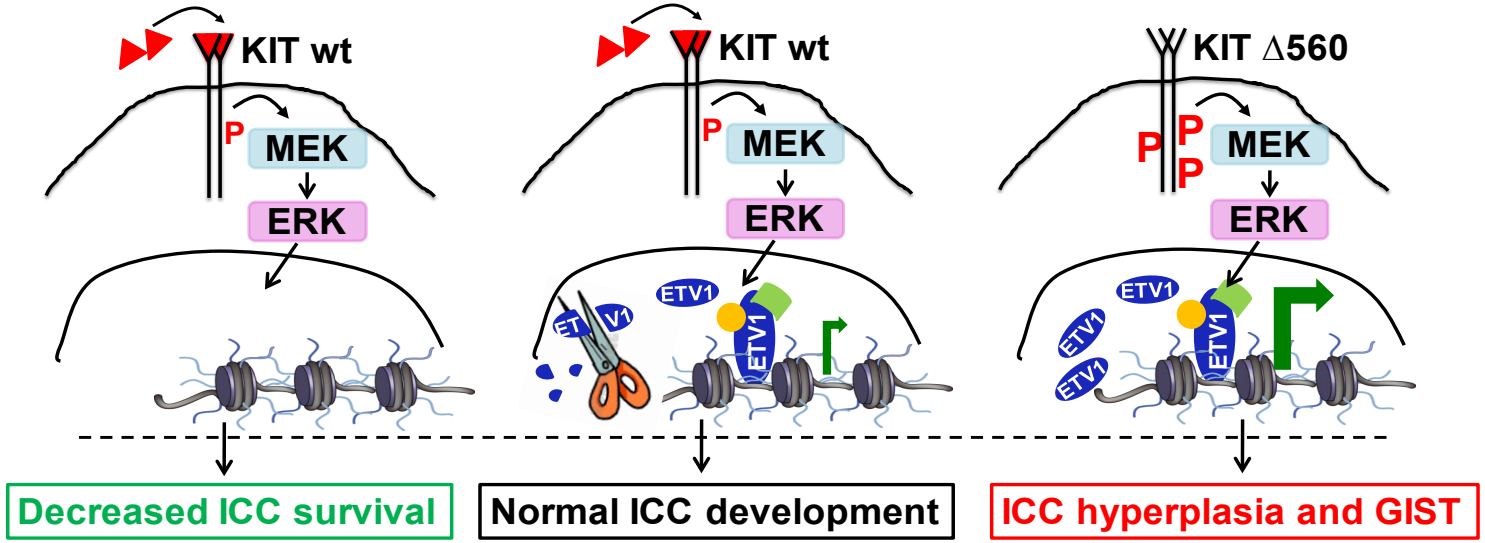
Transcriptional upregulation of KIT by stabilized ETV1

GIST882 cells



Excised 3T3 allograft tumors





Chi, P, Chen, Y et al, Nature 2010; Ran L et al., Cancer Discovery, 2015

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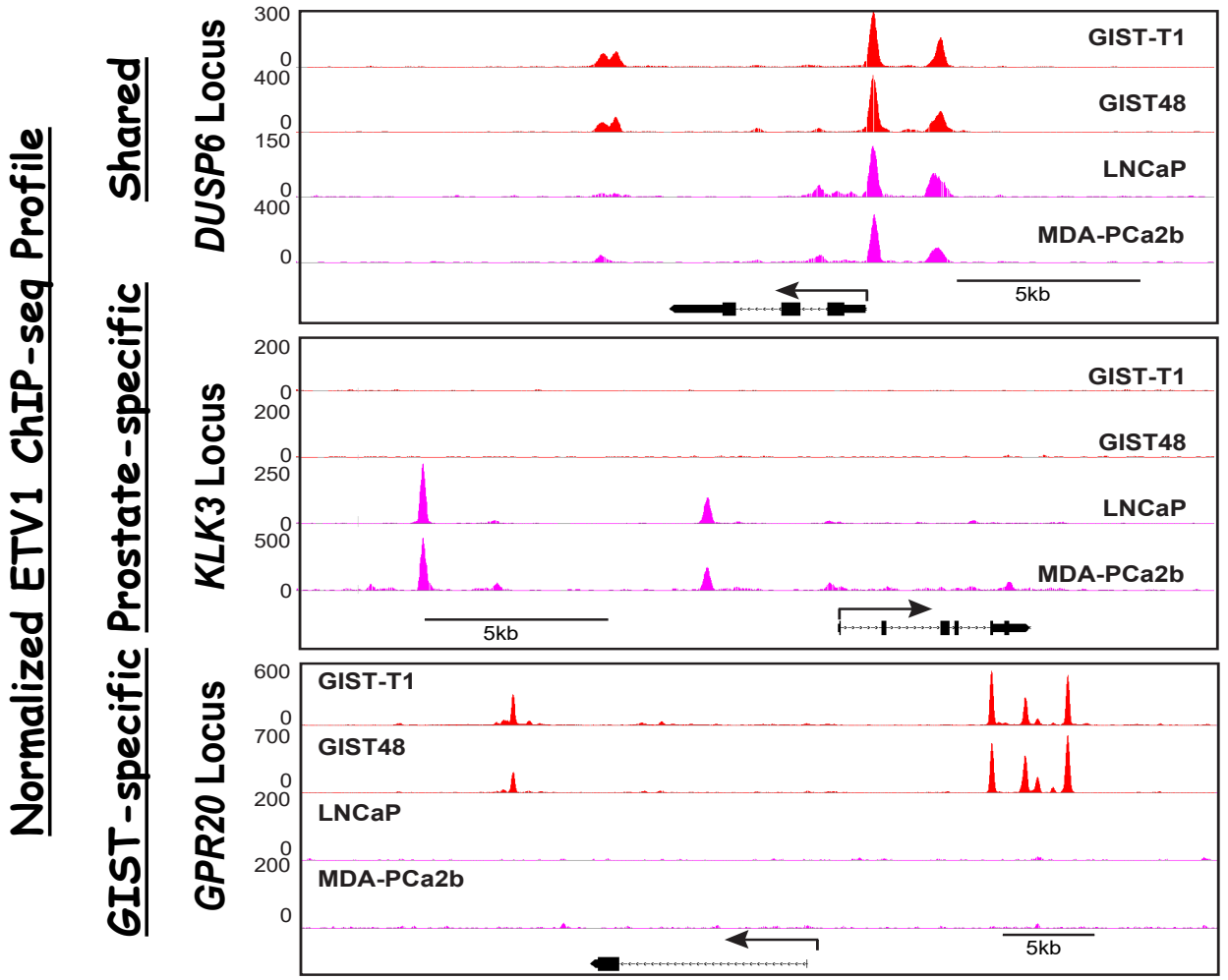
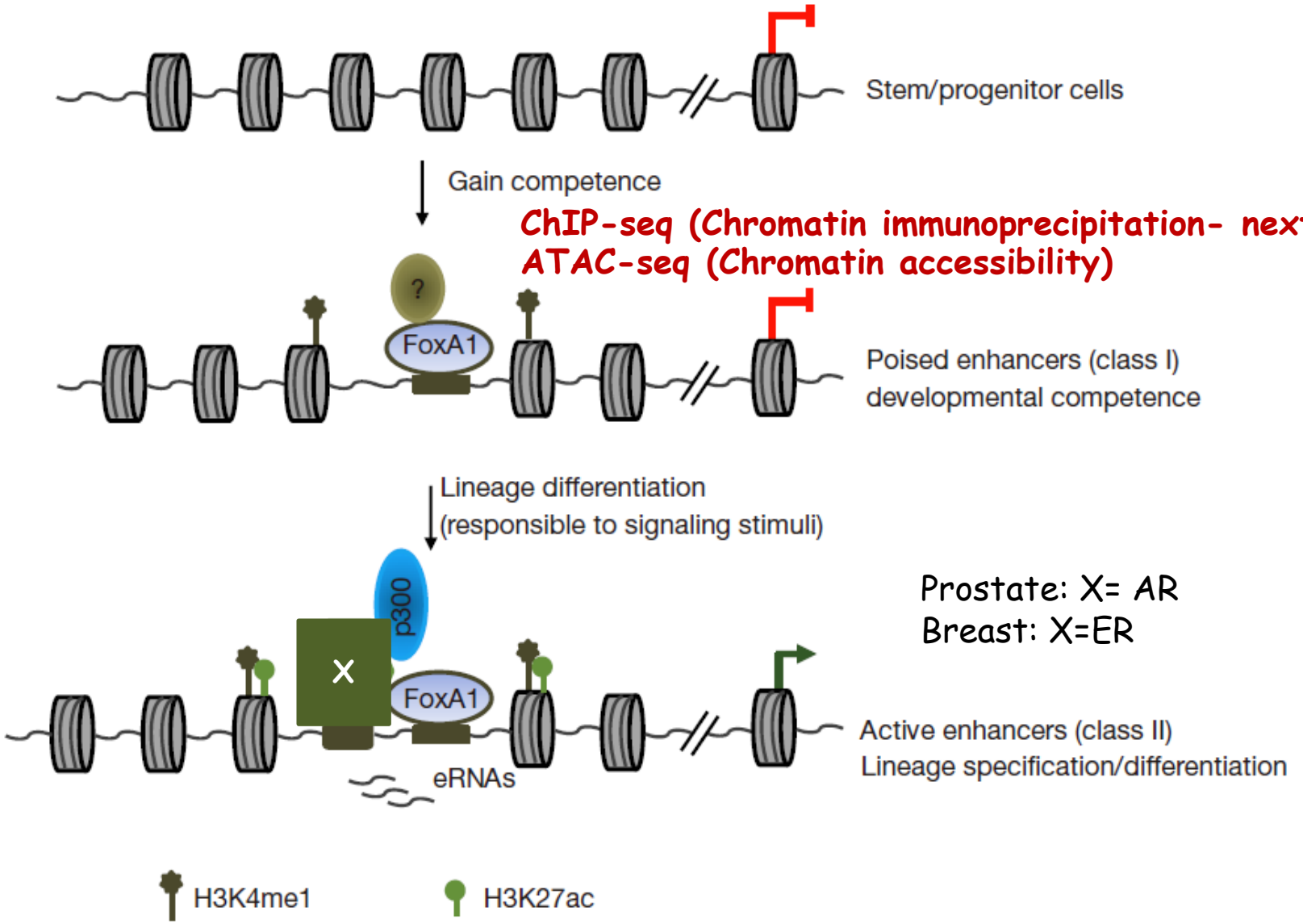
How does ETV1 regulate lineage-specific oncogenic transcriptome in cancer?

- GIST (ICC/GIST lineage-specific)
- Prostate Cancer (Prostate lineage-specific)



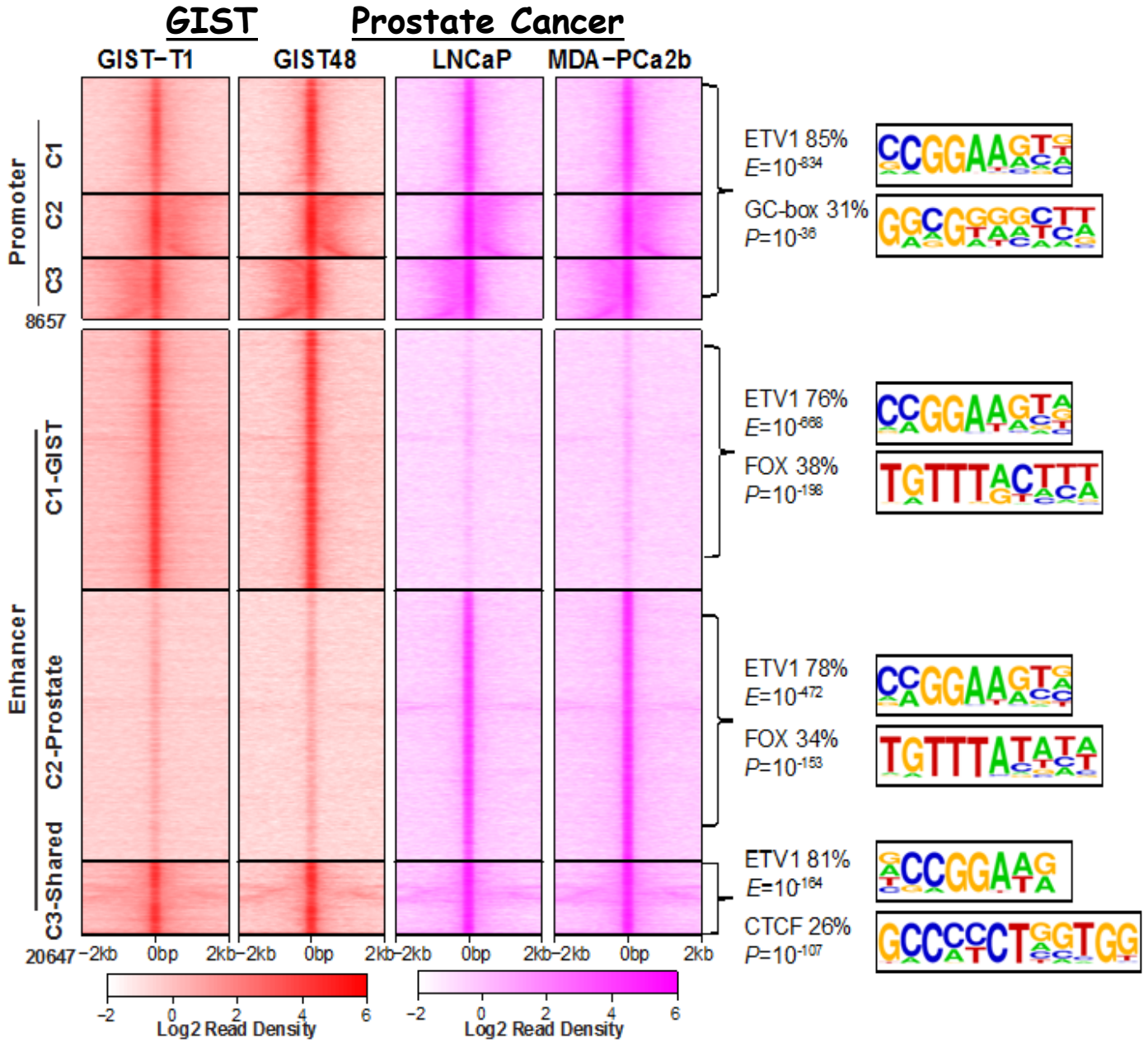
Chi P, Chen Y, et al, Nature, 2010; Chen Y, Chi P, et al., Nat Medicine 2013; Ran L, Chen Y, et al, Cancer Discovery 2015, 2018

Enhancers define cell lineage



Adapted from Li and Huang, 2016

ETV1 cistrome analysis



Ran L, Chen Y, et al., Cancer Discovery 2018

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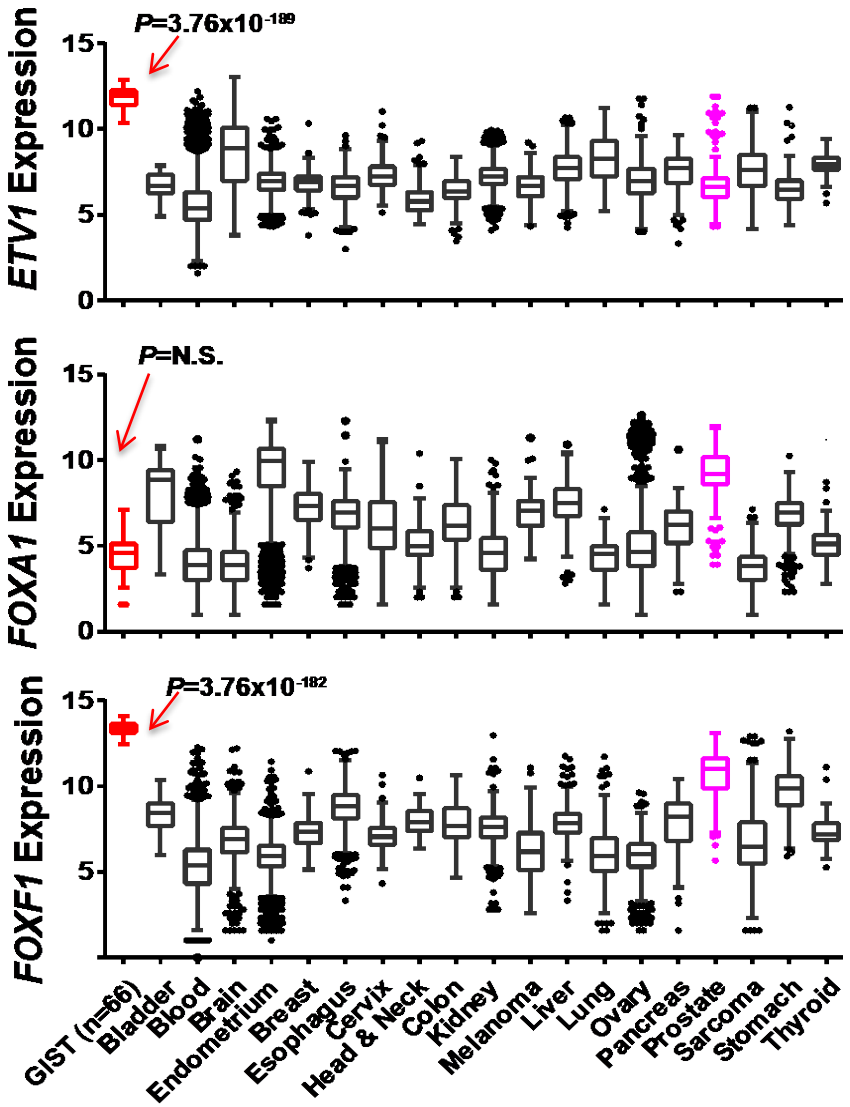
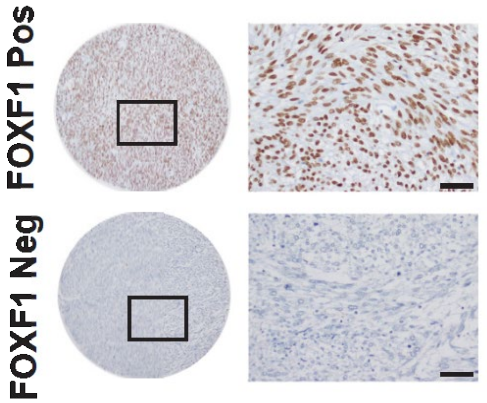
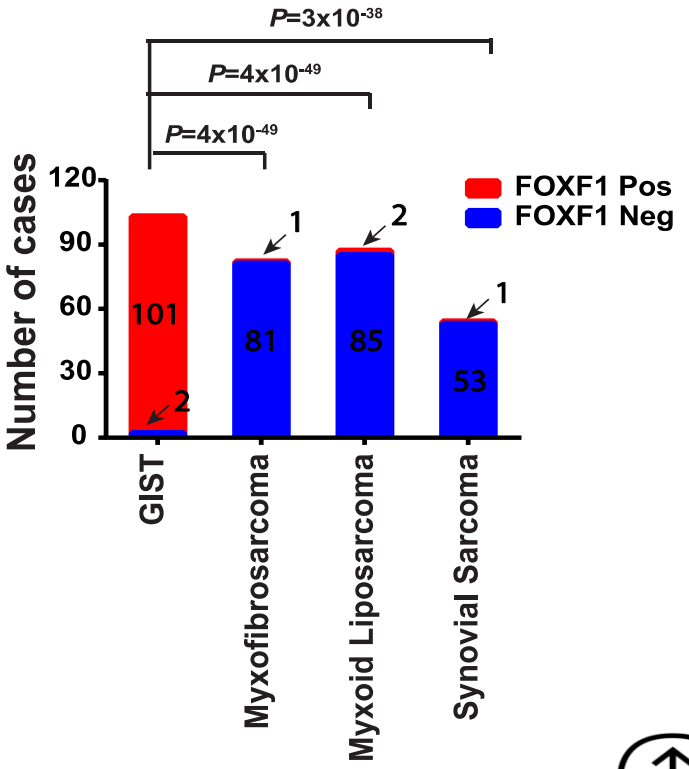
FOXF1 is most differentially and highly expressed in GIST







Ran L, Chen Y, et al., Cancer Discovery 2018

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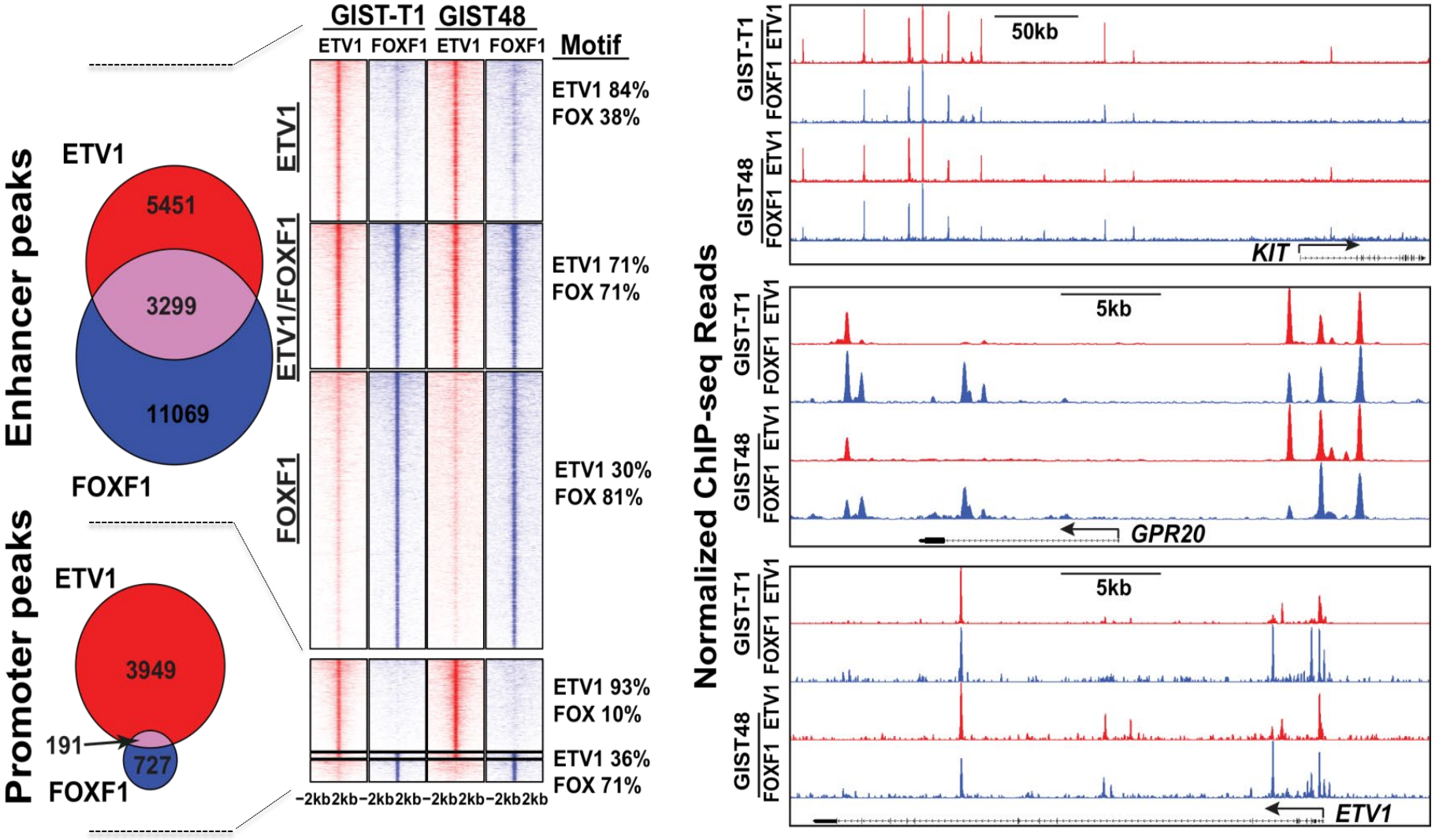
FOXF1 and ETV1 co-localizes at enhancers in GIST



Ran L, Chen Y, et al., Cancer Discovery 2018

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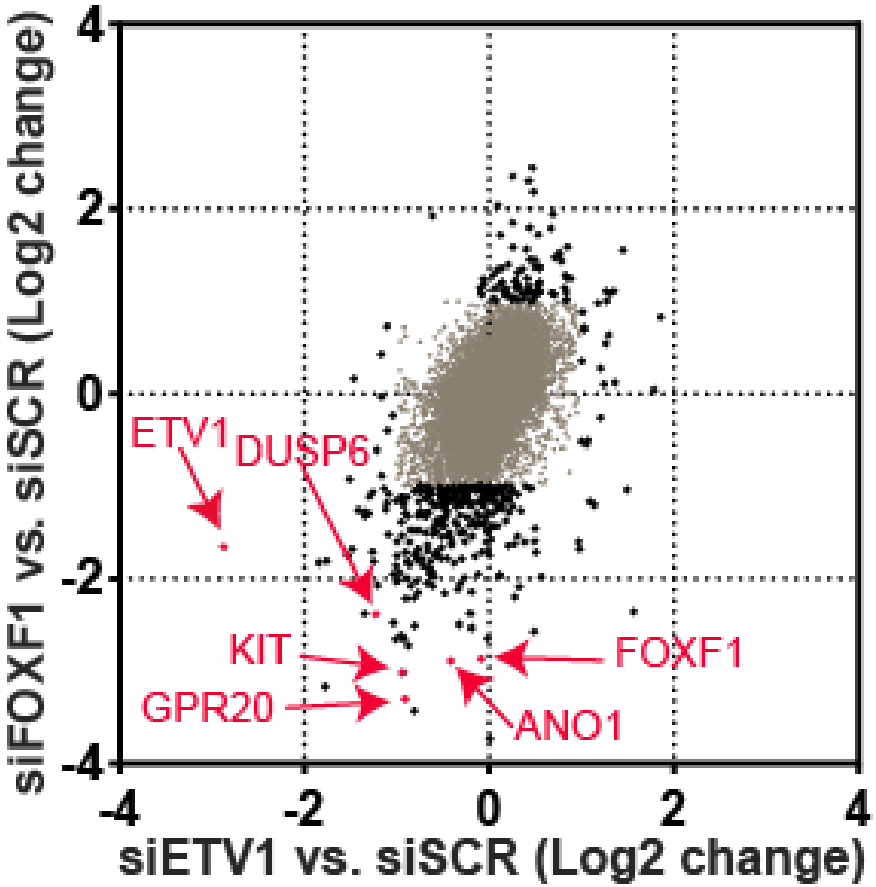
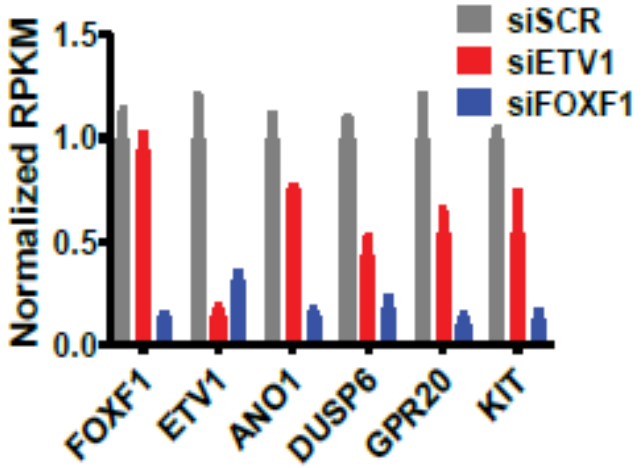
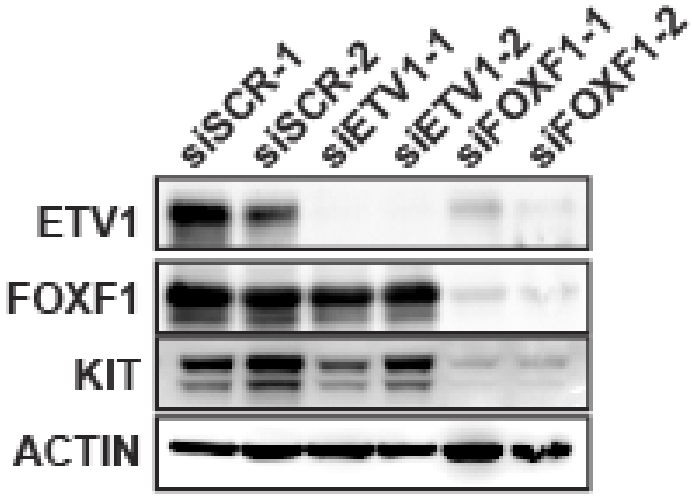
FOXF1 regulates ETV1 and KIT expression







Ran L, Chen Y, et al., Cancer Discovery 2018

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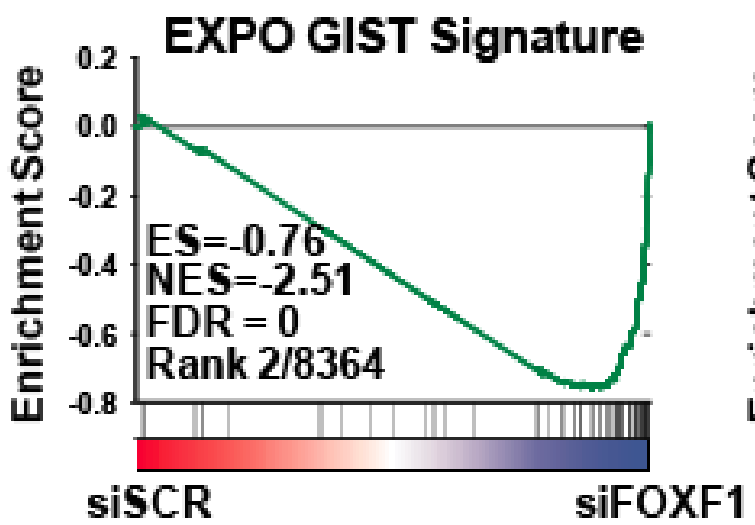
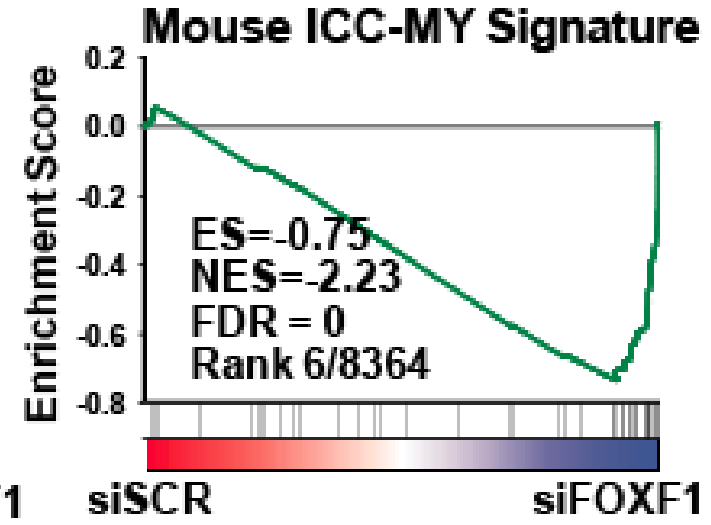
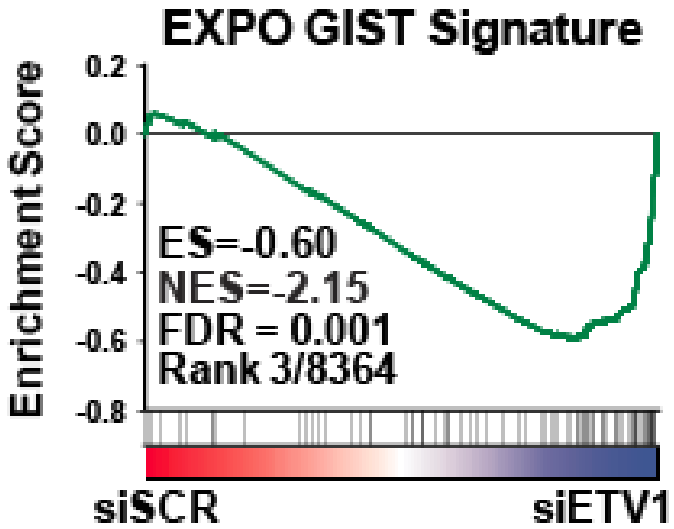
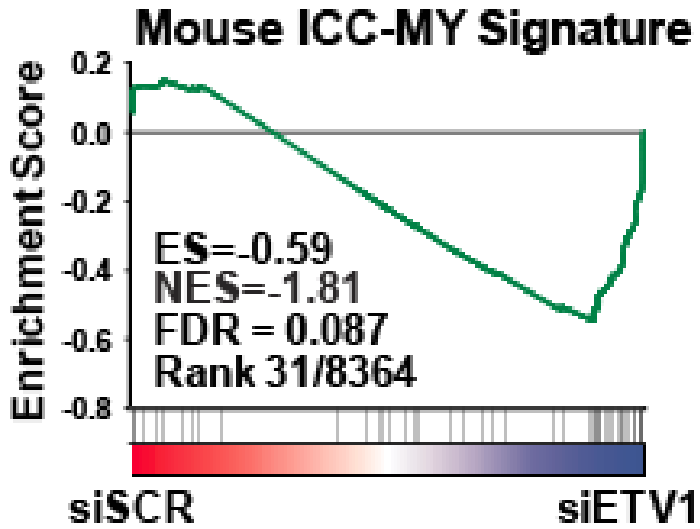
FOXF1 regulates ICC/GIST transcriptome









Ran L, Chen Y, et al., Cancer Discovery 2018

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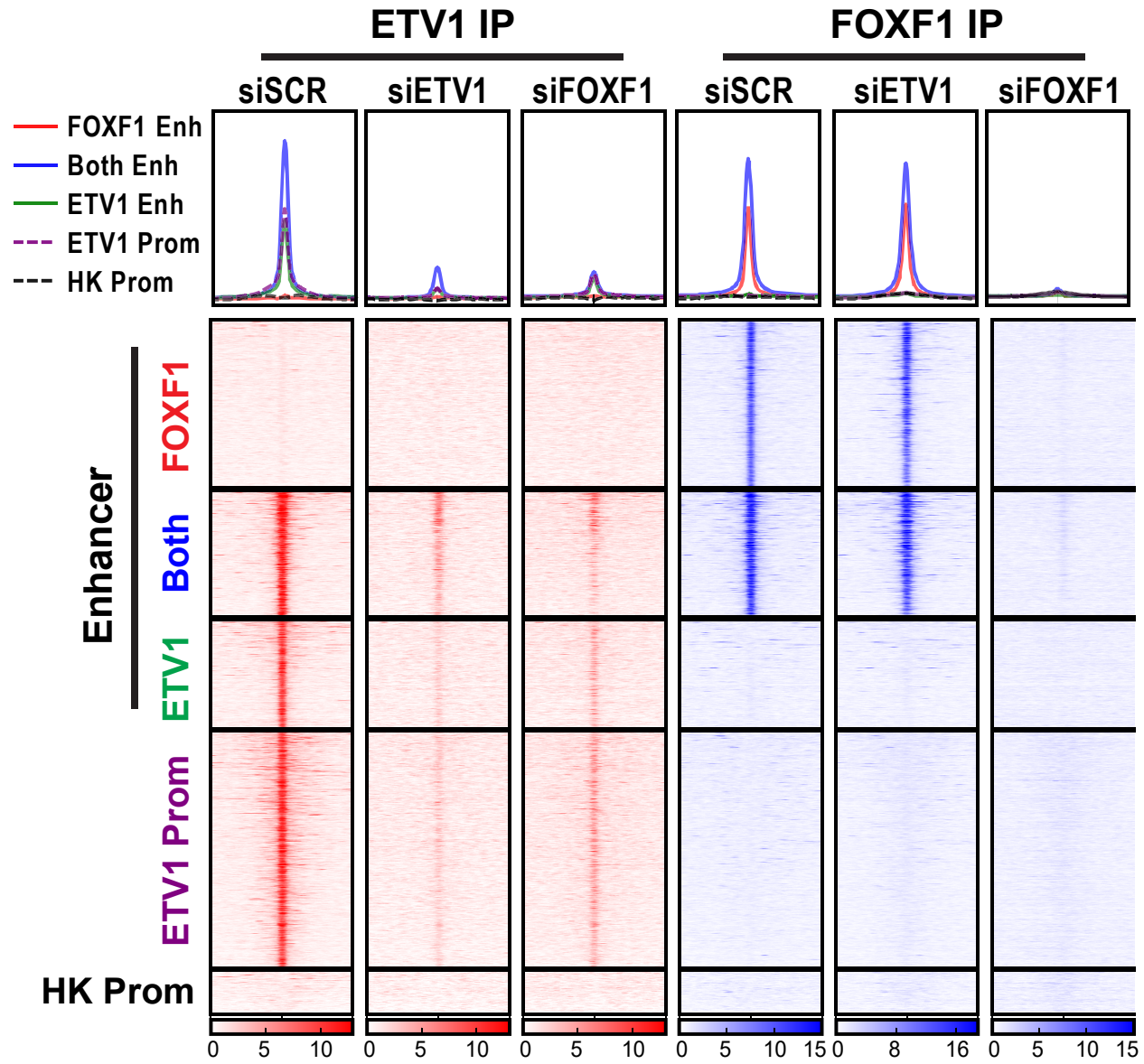
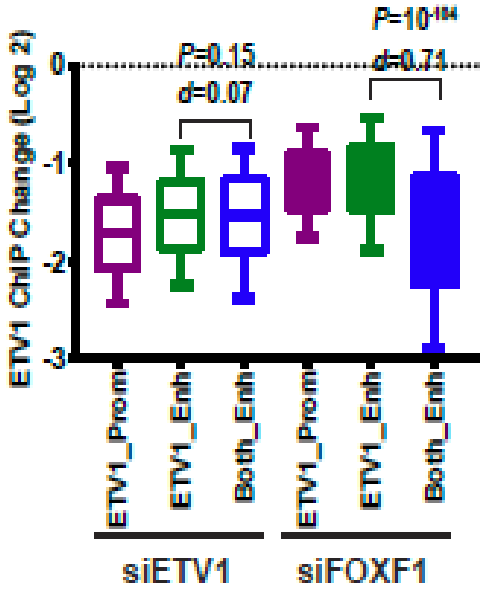
FOXF1 regulates ETV1 cistrome in GIST





Ran L, Chen Y, et al., Cancer Discovery 2018

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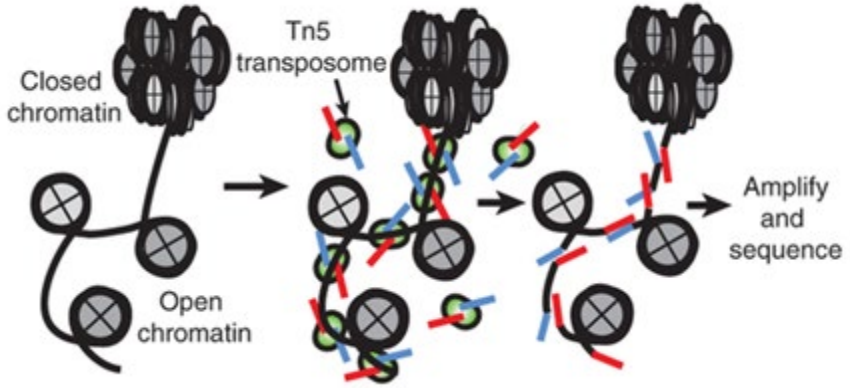
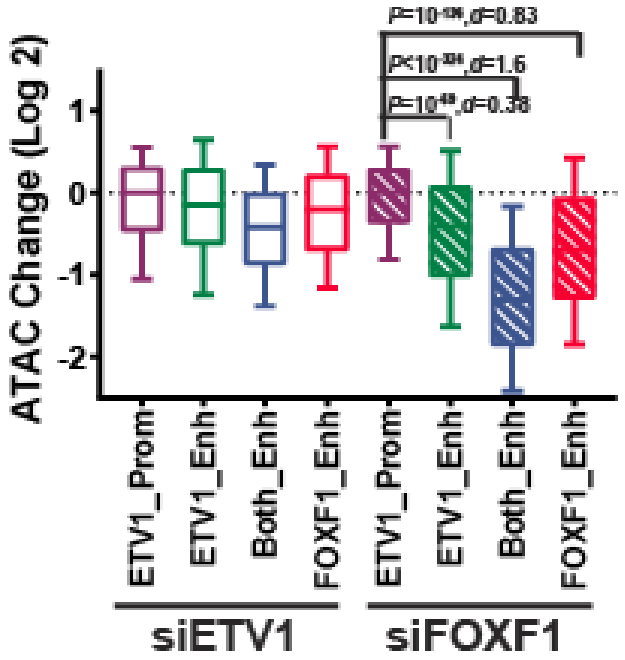
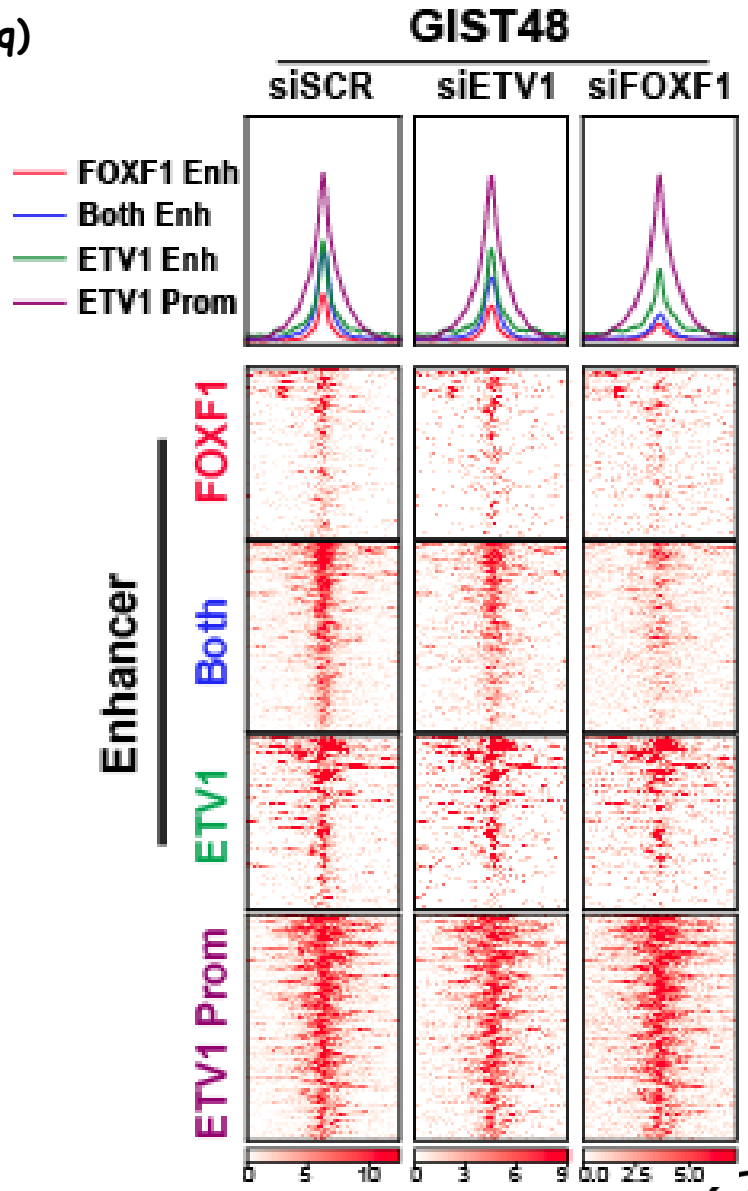
FOXF1 regulates chromatin accessibility in GIST

Assay for Transposase-Associated Chromatin (ATAC-seq)







Buenrostro et. al. 2013 Nat Methods

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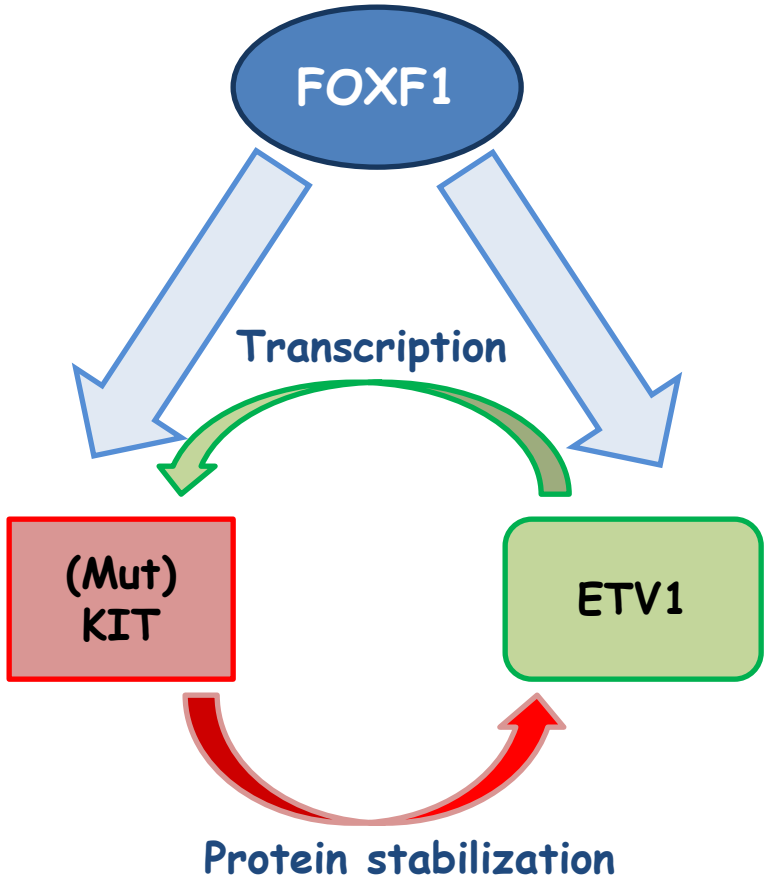
FOXF1 functions as a "pioneer factor" that enforces lineage-dependency in GIST pathogenesis



Multilevel transcriptional regulation

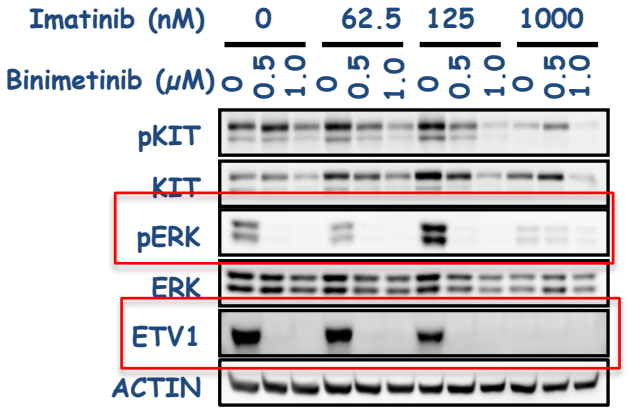
- Direct transcriptional activation of KIT
- Transcriptional regulation of masterregualtor-ETV1 (Cistrome regulation, Chromatin accessibility…)

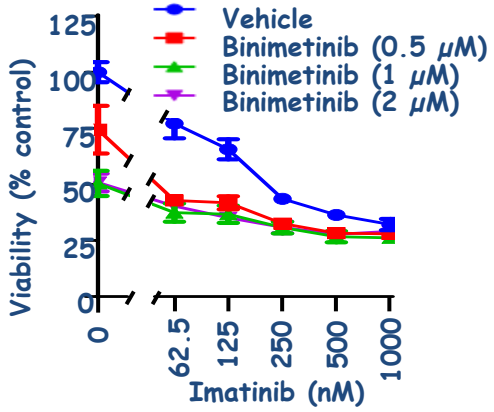
GIST-High lineage addiction

- dependent on KIT/PDGFRA signaling
- <1% dedifferentiation in treatment-refractory GIST

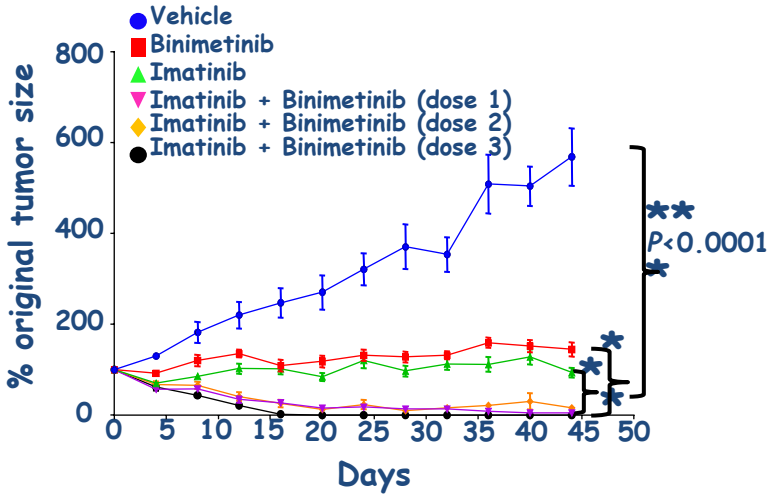
Synergy of combined MAPK and KIT pathway inhibition

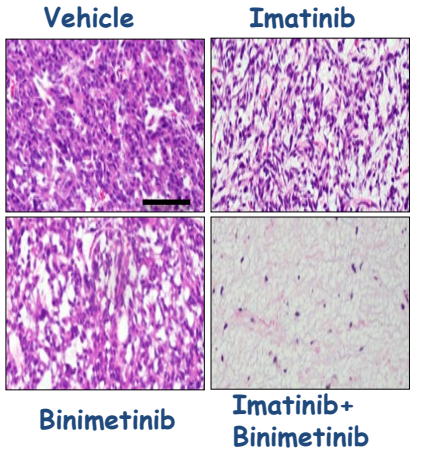
GIST882

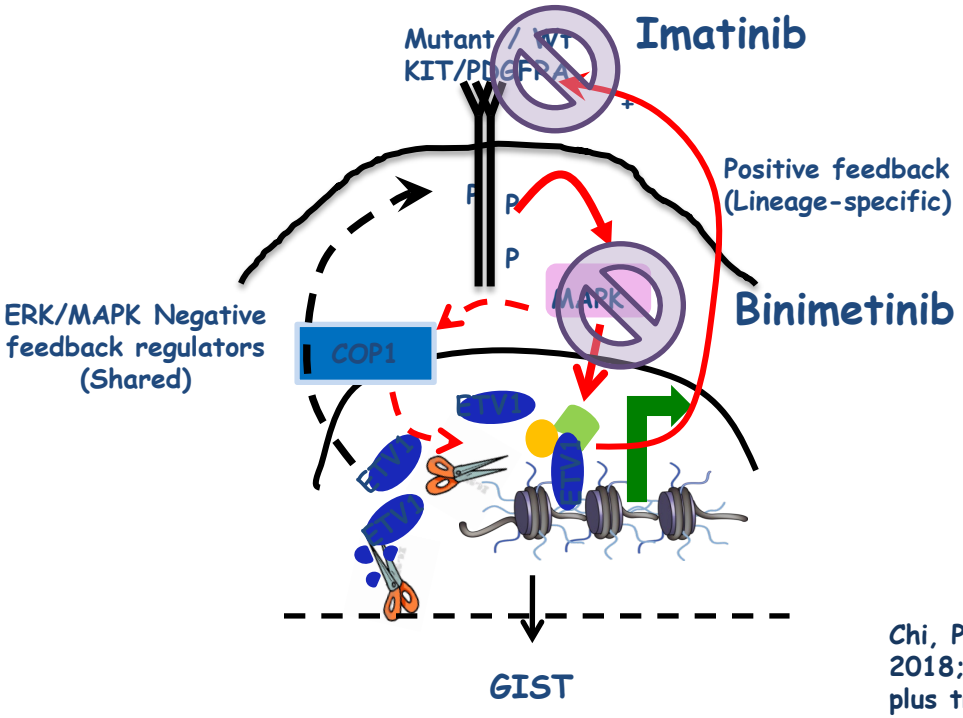




GIST882 xenografts







Advantage of targeting lineage dependence:

- Bypasses multiple upstream resistance mechanisms
- Break the positive feedback circuit of ETV1/KIT-target KIT expression regardless of mutations
- Block early adaptation and forestall resistance development and induces enhanced depth of cytotoxicity

A phase Ib/II trial of imatinib plus binimetinib in advanced GIST
- Positive trial ! Combination is also effective in SDH-deficient GIST

Chi, P, Chen, Y et al, Nature 2010; Ran L et al., Cancer Discovery, 2015, 2018; Xie et al, JCI 2018; Gupta A et al., Mol Cancer Ther, 2021 (ripretinib plus trametinib); Chi P et al., CCR 2022; Chi P et al., JCO 2022.

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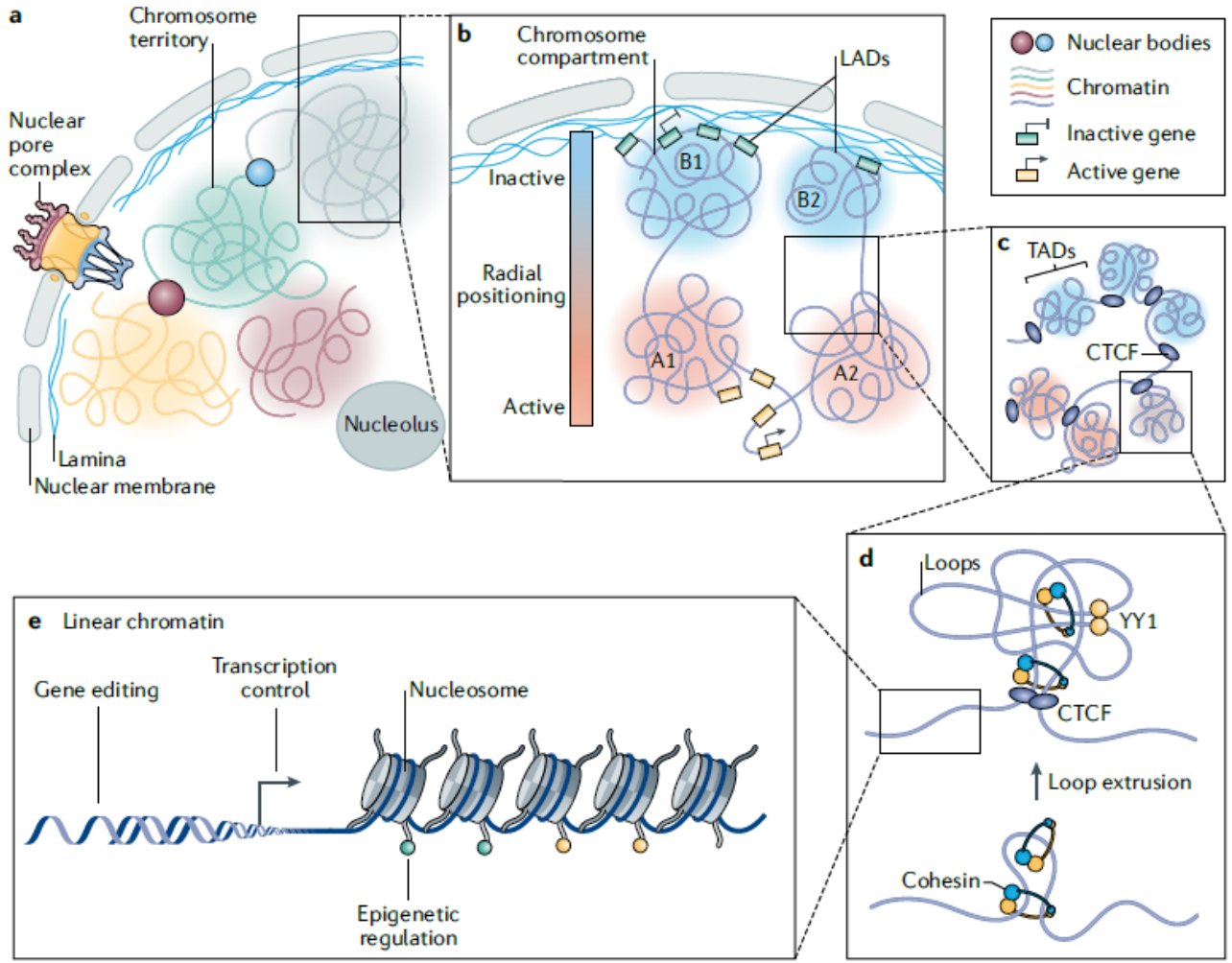
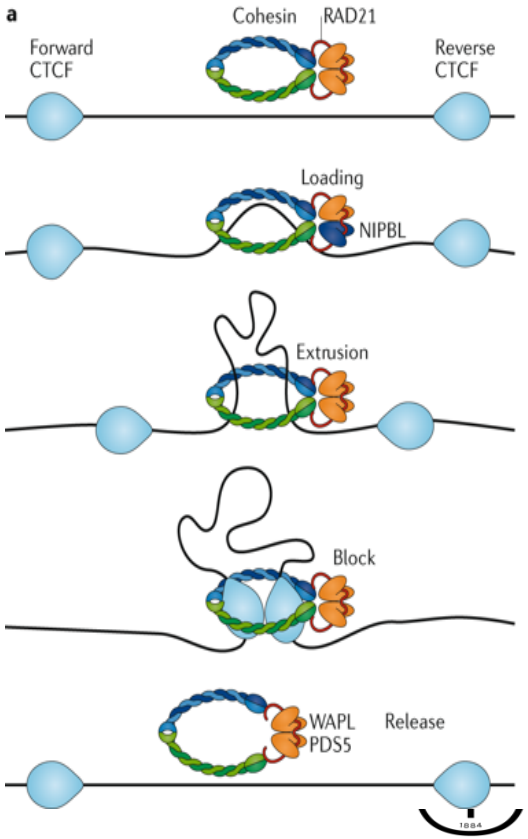
Hierarchical organization of the 3D genome in the nucleus



Compartments: ~10-100Mb
TADs: ~200kb-2Mb
Loops: ~10kb-1Mb

Loop extrusion and TAD organization



Wang H., Han M., Qi LS, Nat Rev Genetics, 2021

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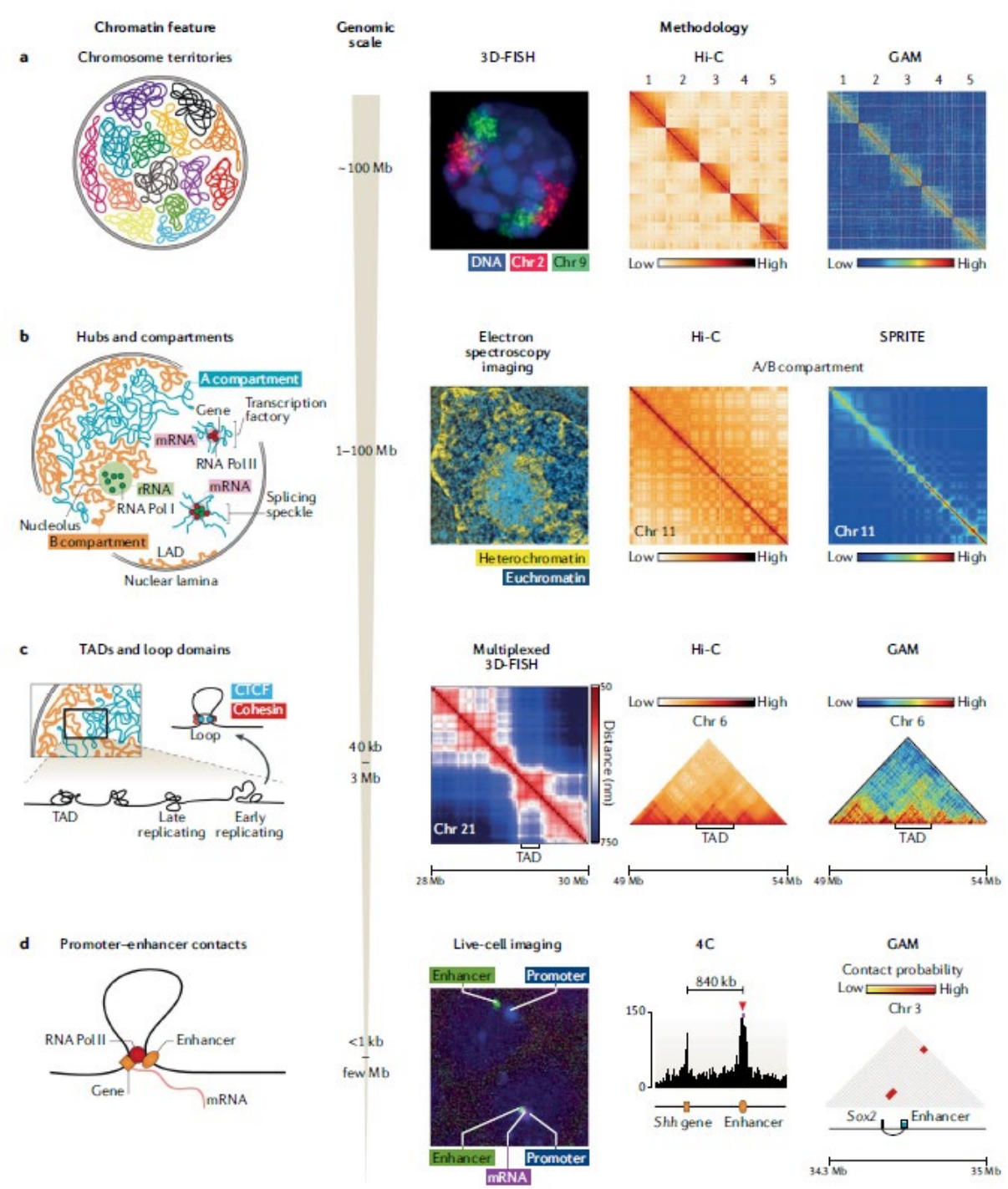
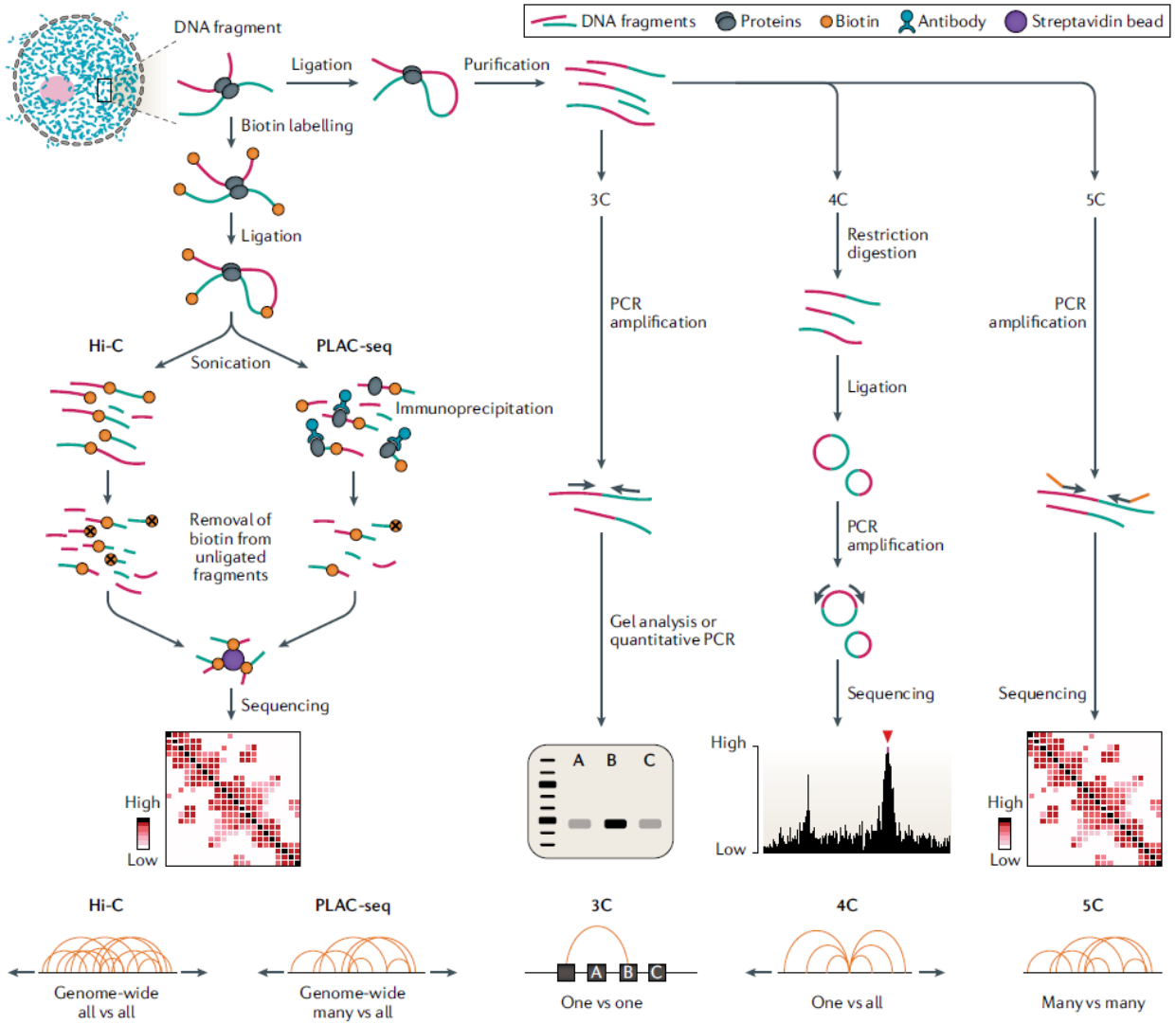
Methods for mapping 3D chromosome architecture





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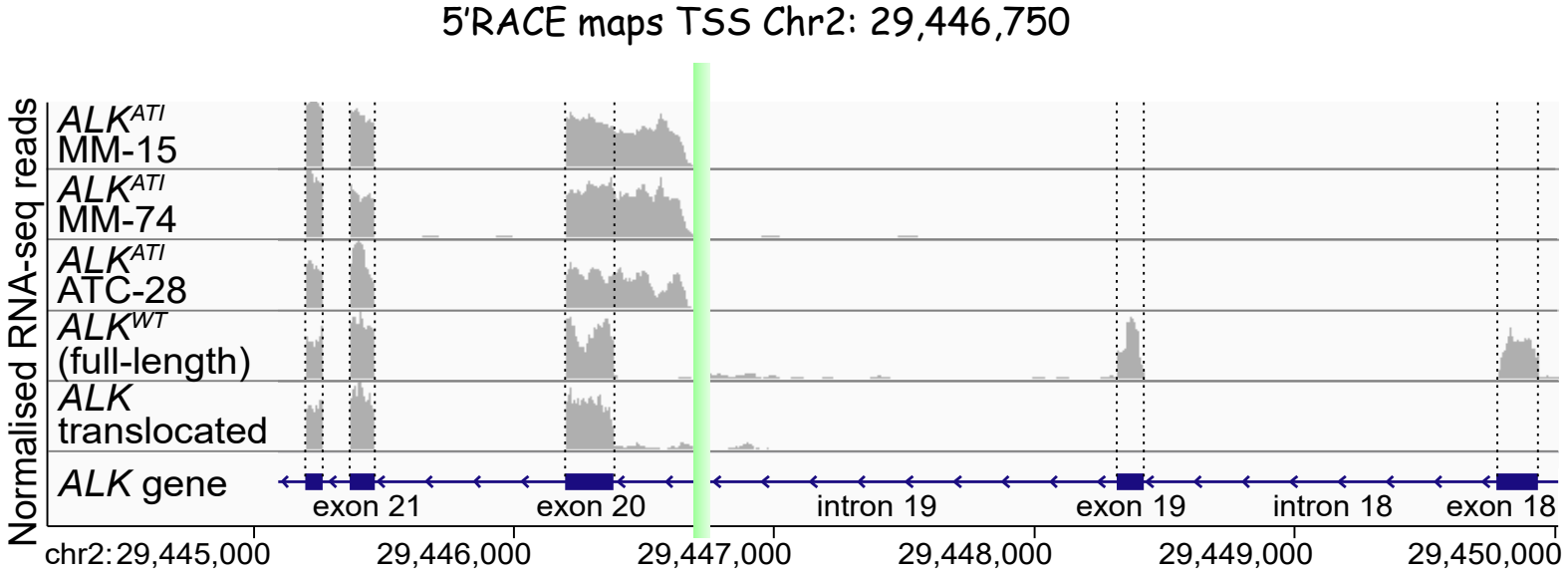
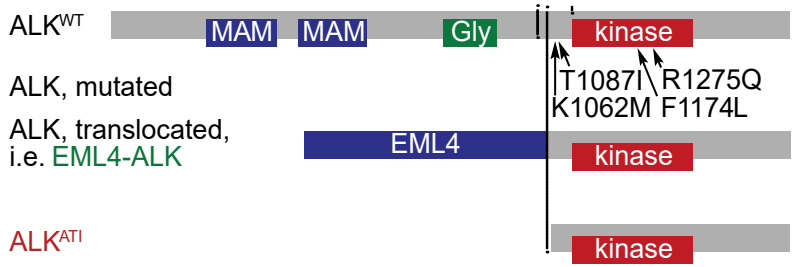
Kempfer R and Pombo A, Nat Rev. Genetics, 2020

A novel *ALK* variant through alternative transcription initiation (alternative promoter usage)





Functional:
-drives oncogenesis
-respond to ALK inhibitors

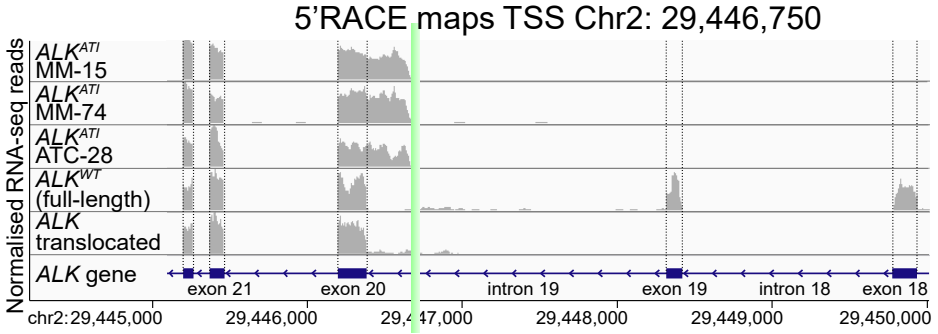
TCGA RNA-seq

Type	ALK ATI	Total # of cases	%
Skin cutaneous melanoma (SKCM)	38	334	11.34
Lung adenocarcinoma (LUAD)	3	470	0.64
Lung squamous cell carcinoma (LUSC)	1	482	0.20
Kidney renal clear cell carcinoma (KIRC)	2	480	0.42
Breast invasive carcinoma (BRCA)	1	988	0.10
Thyroid carcinoma (THCA)	0	482	0.00
Glioblastoma multiforme (GBM)	0	153	0.00
Brain lower grade glioma (LGG)	0	271	0.00
Bladder urothelial carcinoma (BLCA)	0	182	0.00
Prostate adenocarcinoma (PRAD)	0	195	0.00
Uterine corpus endometrial carcinoma (UCEC)	0	118	0.00
Kidney chromophobe (KICH)	0	66	0.00
Colorectal adenocarcinoma (COADREAD)	0	316	0.00
Ovarian carcinoma (OV)	0	261	0.00
Head and neck squamous cell carcinoma (HNSC)	0	303	0.00

Wiesner T et al., Nature 2015

Memorial Sloan Kettering Cancer Center

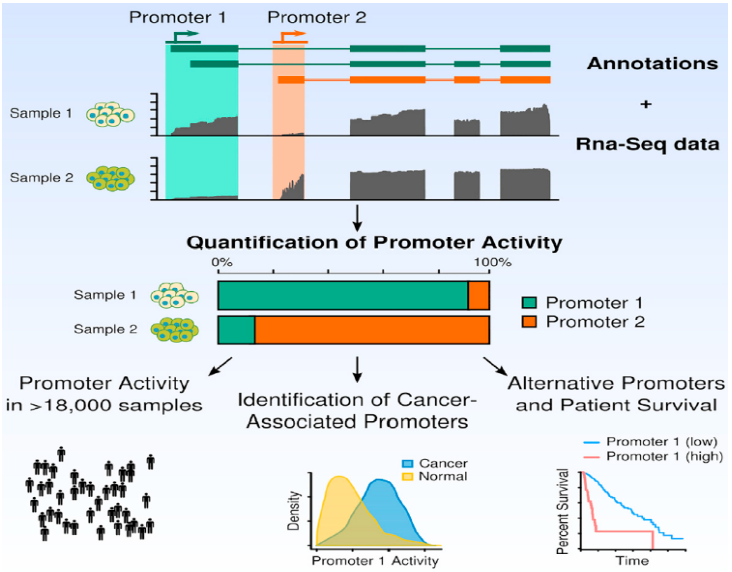
Alternative promoter usage in cancer



Wiesner T et al., Nature 2015

Alternative transcription initiation leads to expression of a novel oncogenic *ALK* isoform in cancer

- *ALK*^{ATI} present in 11% of melanomas and sporadically other cancers
- *ALK*^{ATI} transcriptional activation is regulated by **epigenetic mechanisms**



Demircioglu D., et al, Cell 2019

Pan-cancer transcriptome analysis reveals pervasive regulation through **alternative promoters**

- Estimation of promoter activity in 18,468 RNA-seq, 42 cancer types
- Alternative promoters display tissue-specific regulation and impact isoform diversity
- Cancer associated promoters alter the transcriptome independent from gene expression
- Patient-to-patient variation in alternative promoter is associated with survival

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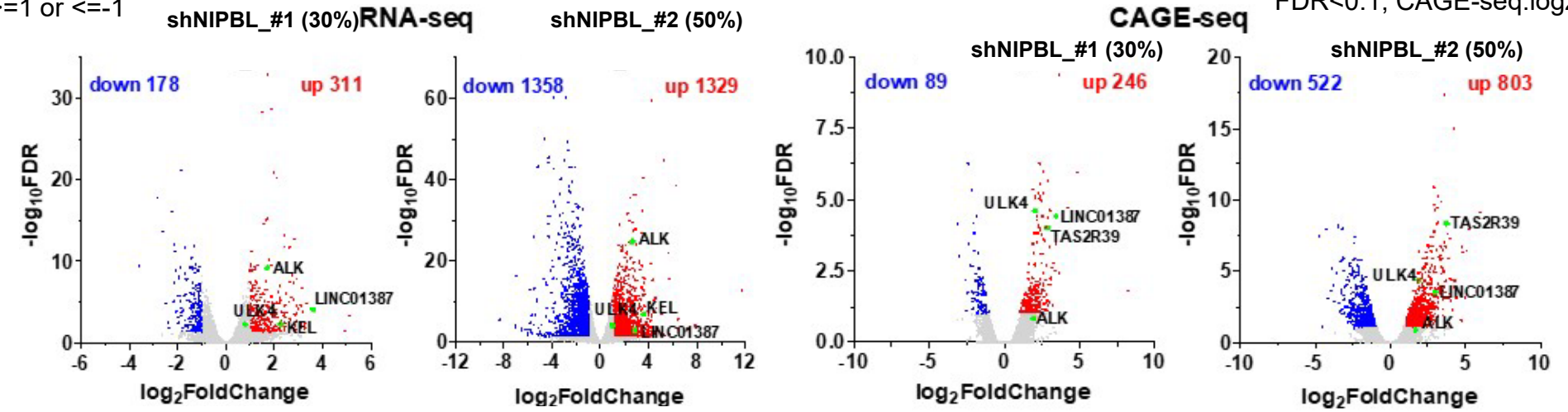
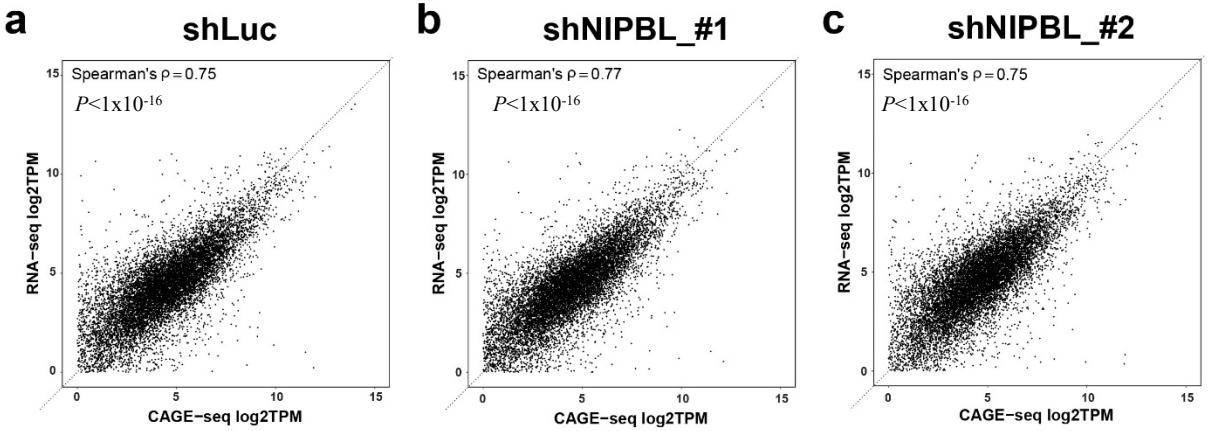
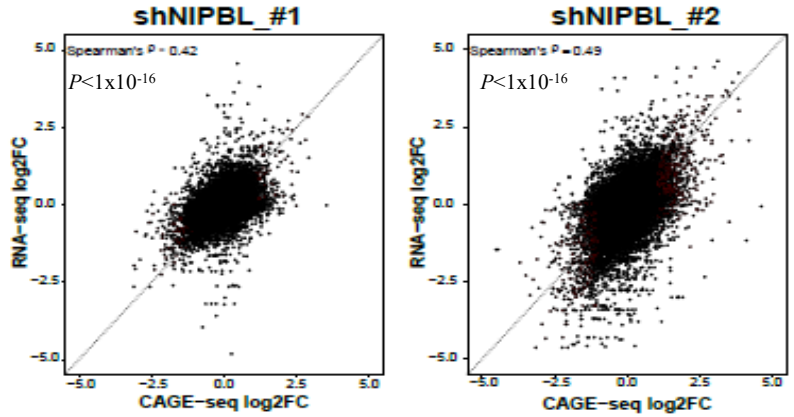
Haploinsufficiency of *NIPBL* leads to global alternative promoter usage

FDR<0.05; log2FC>=1 or <=-1



FDR<0.1; CAGE-seq.log2FC most >=1, <=-1





Wong E et al., in revision

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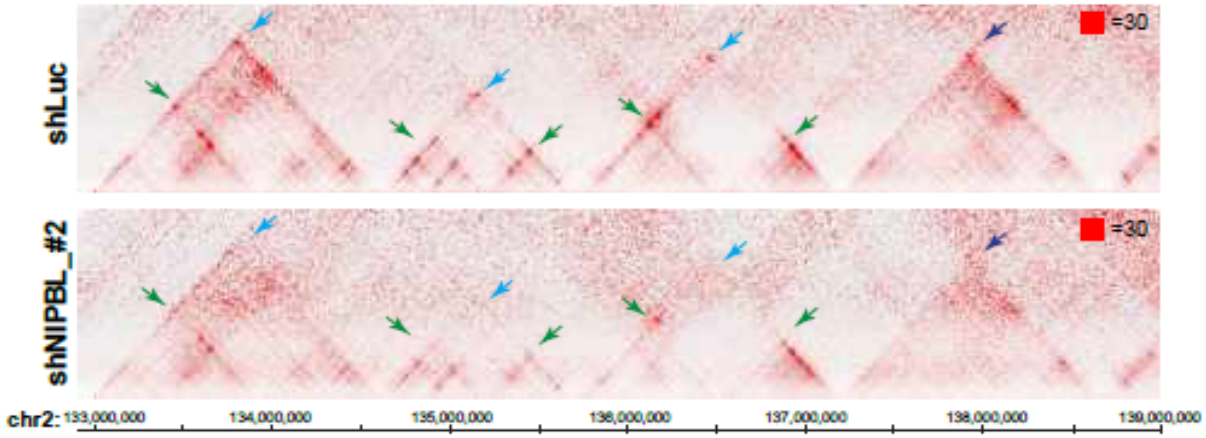
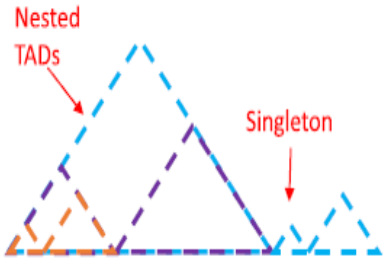
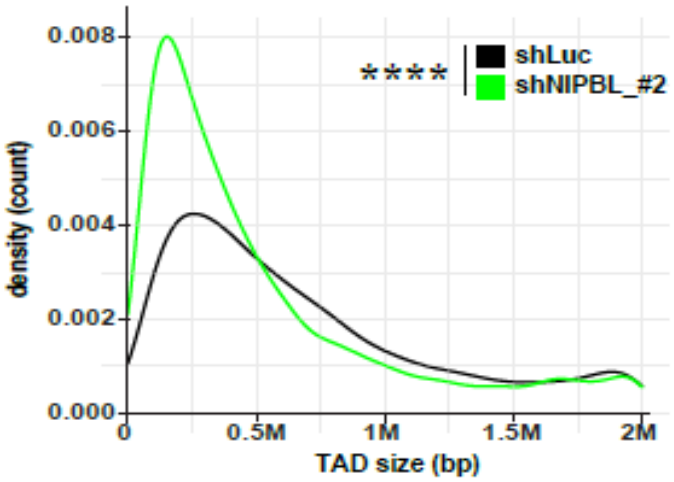
NIPBL perturbation leads to TAD size and insulation decrease and hierarchy loss

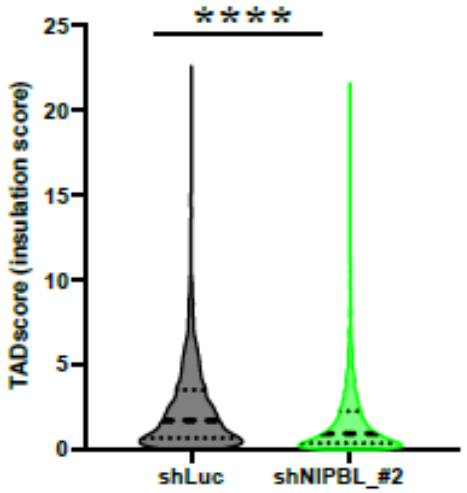
10Kb resolution (Hi-C biological duplicates)

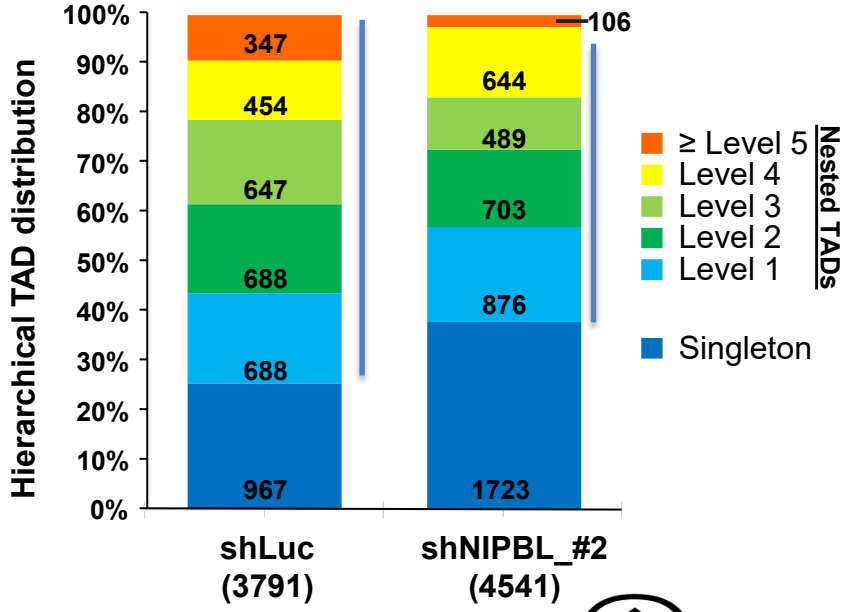




OnTAD (Hierarchy domain structure)
An L et al., 2019







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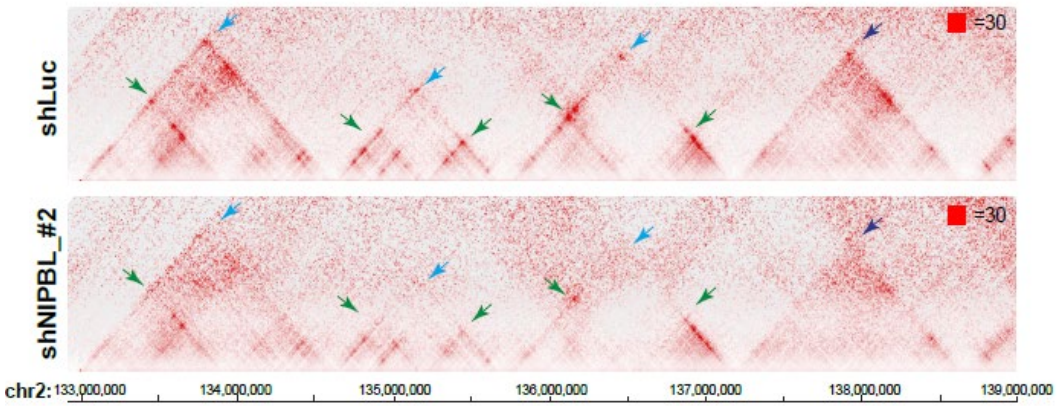
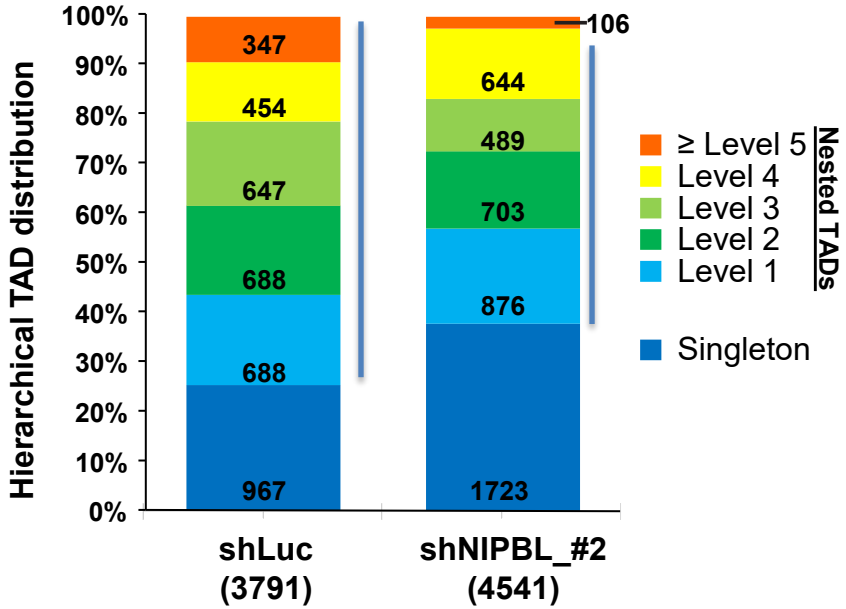
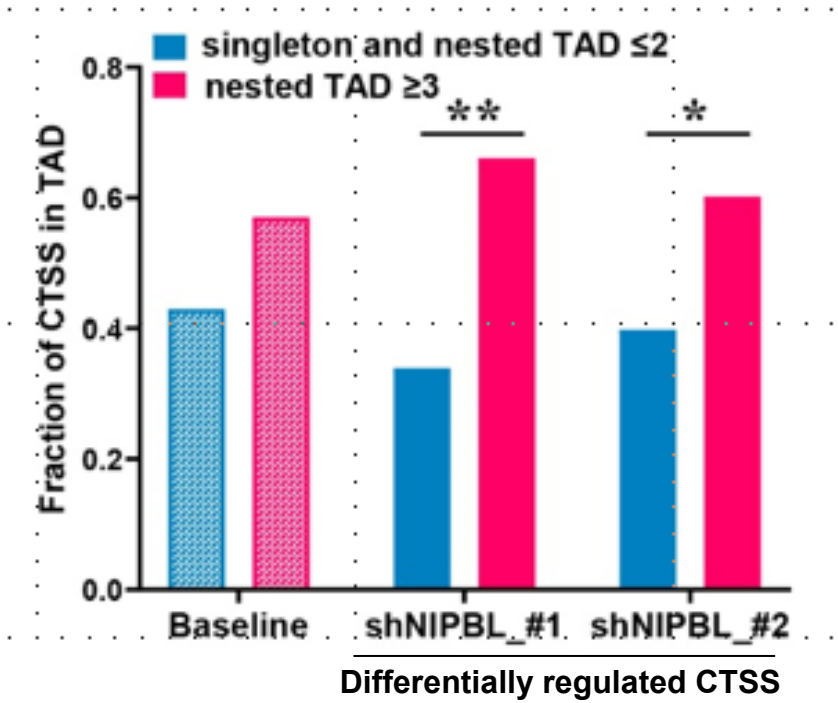
Alternative promoter usage in cancer via perturbation in 3D chromatin hierarchy





Integration of CAGE-seq and TAD hierarchy changes



Nested TAD level	Baseline		Differential in shNIPBL_#1		Differential in shNIPBL_#2	
	Number	Fraction	Number	Fraction	Number	Fraction
Singleton and nested TAD ≤2	17306	0.430	110	0.340	512	0.398
Nested TAD ≥3	22980	0.570	214	0.660	775	0.602
Total CTSS in TAD	40286		324		1287	
P-value			0.0011	**	0.0234	*

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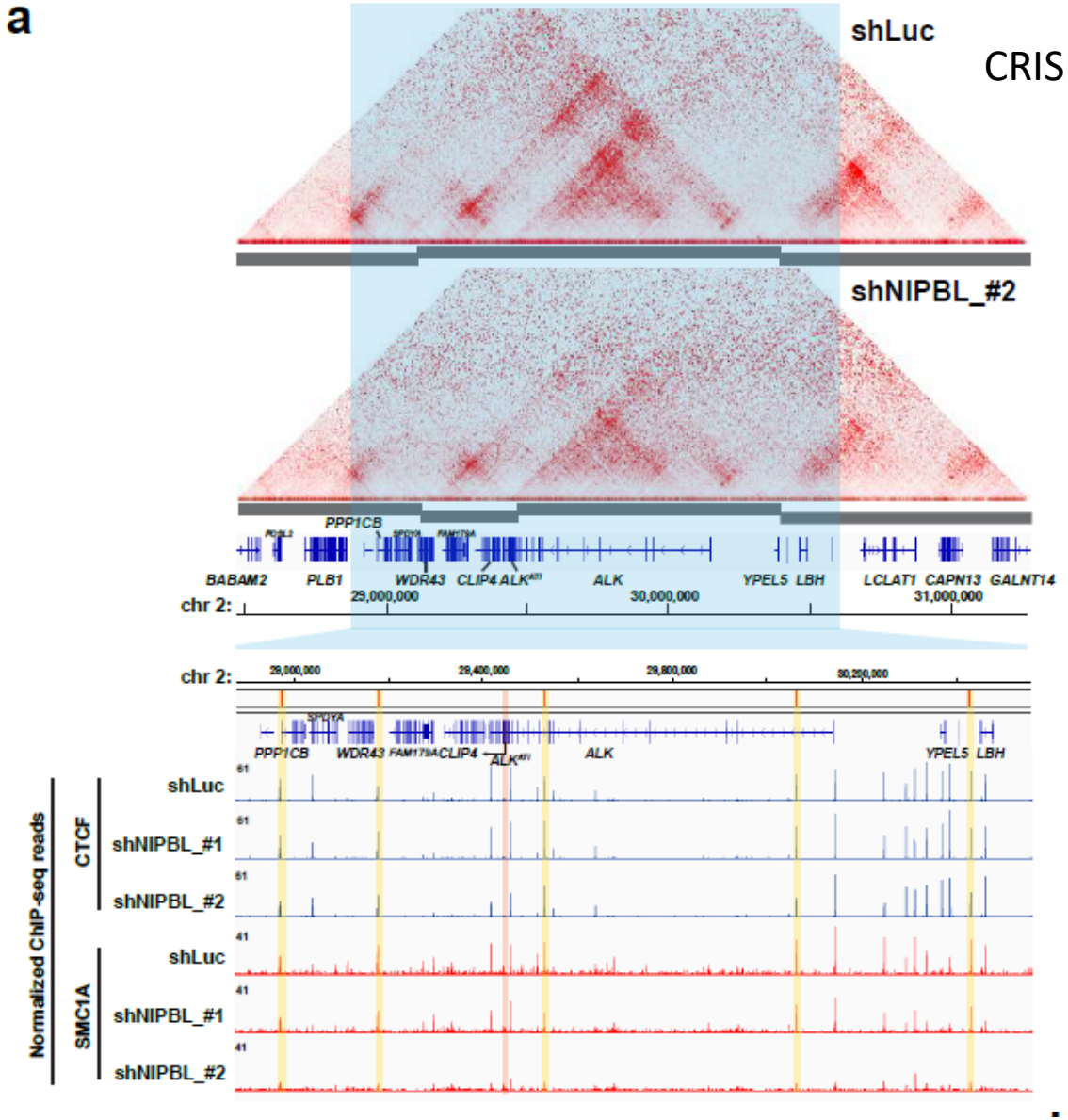
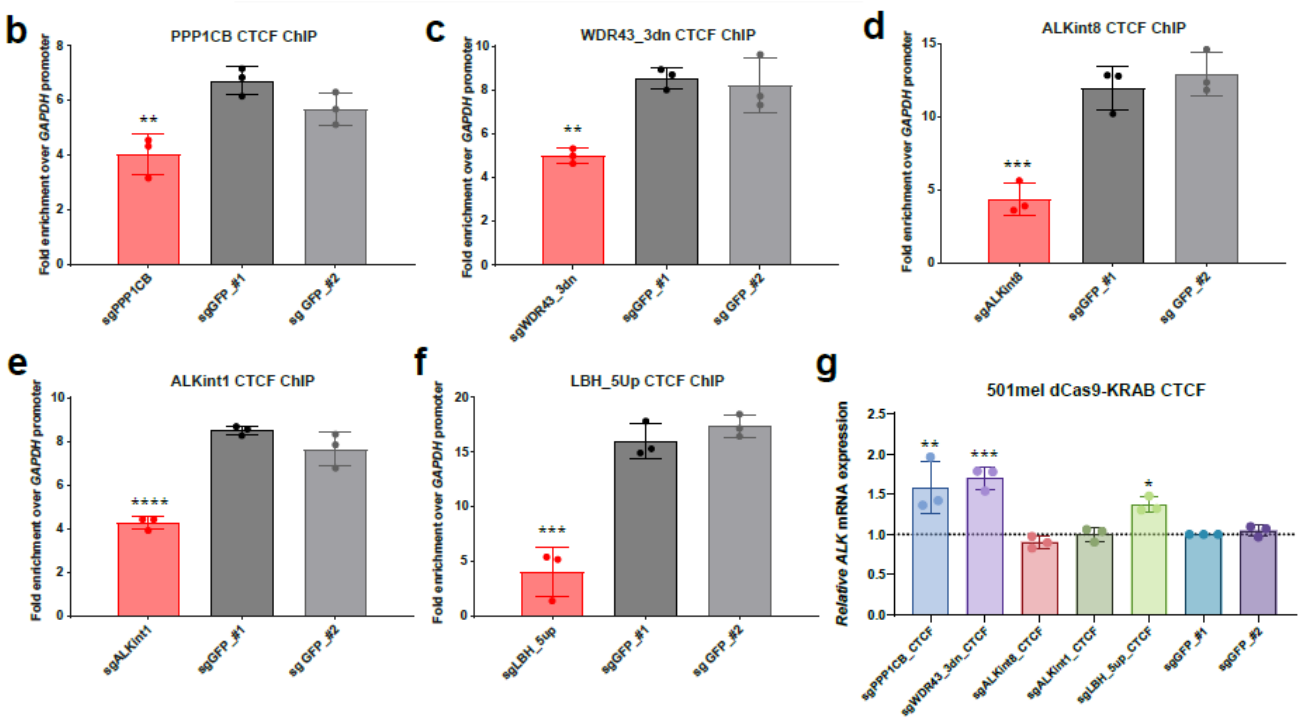
CRISPRi/CRISPR-Cas9-mediated TAD hierarchy loss results in alternative promoter activation



CRISPRi: dCas9-KRAB-mediated blocking of CTCF binding at TAD boundary



CRISPR/Cas9 of WDR43 CTCF sites similar observation

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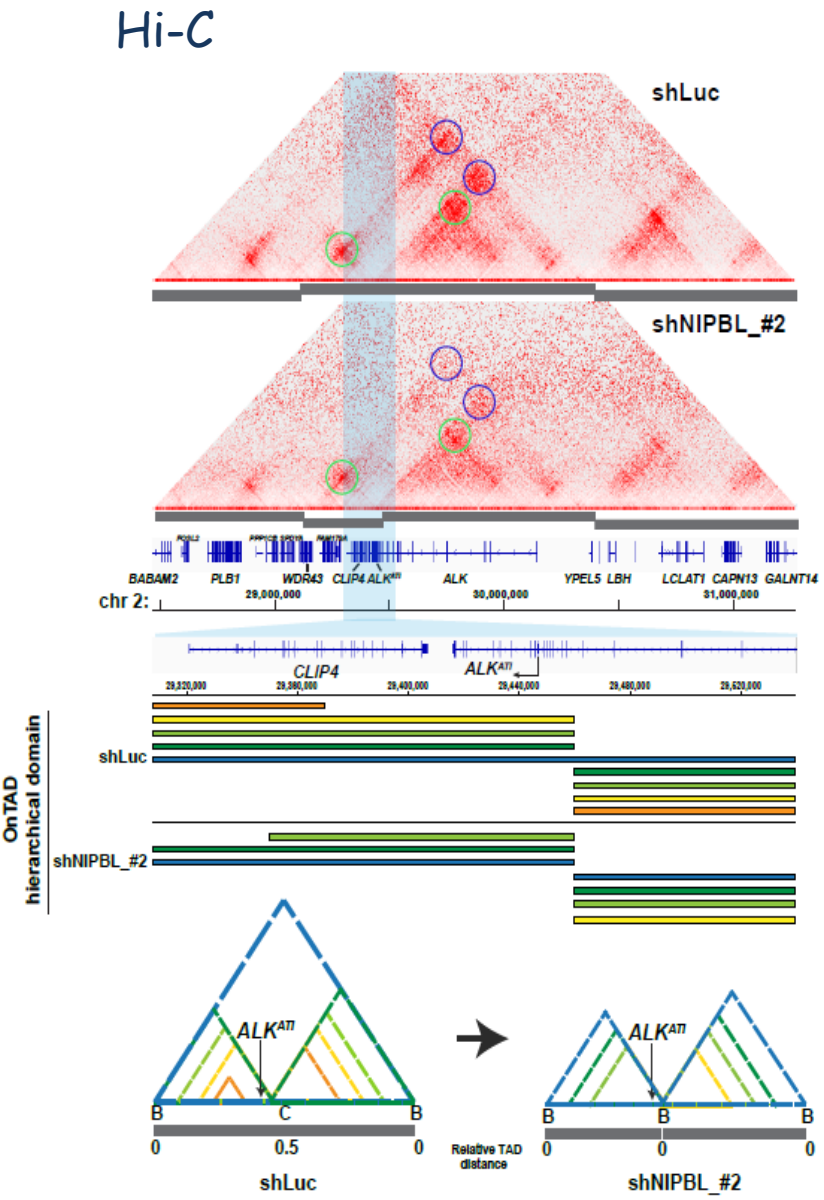
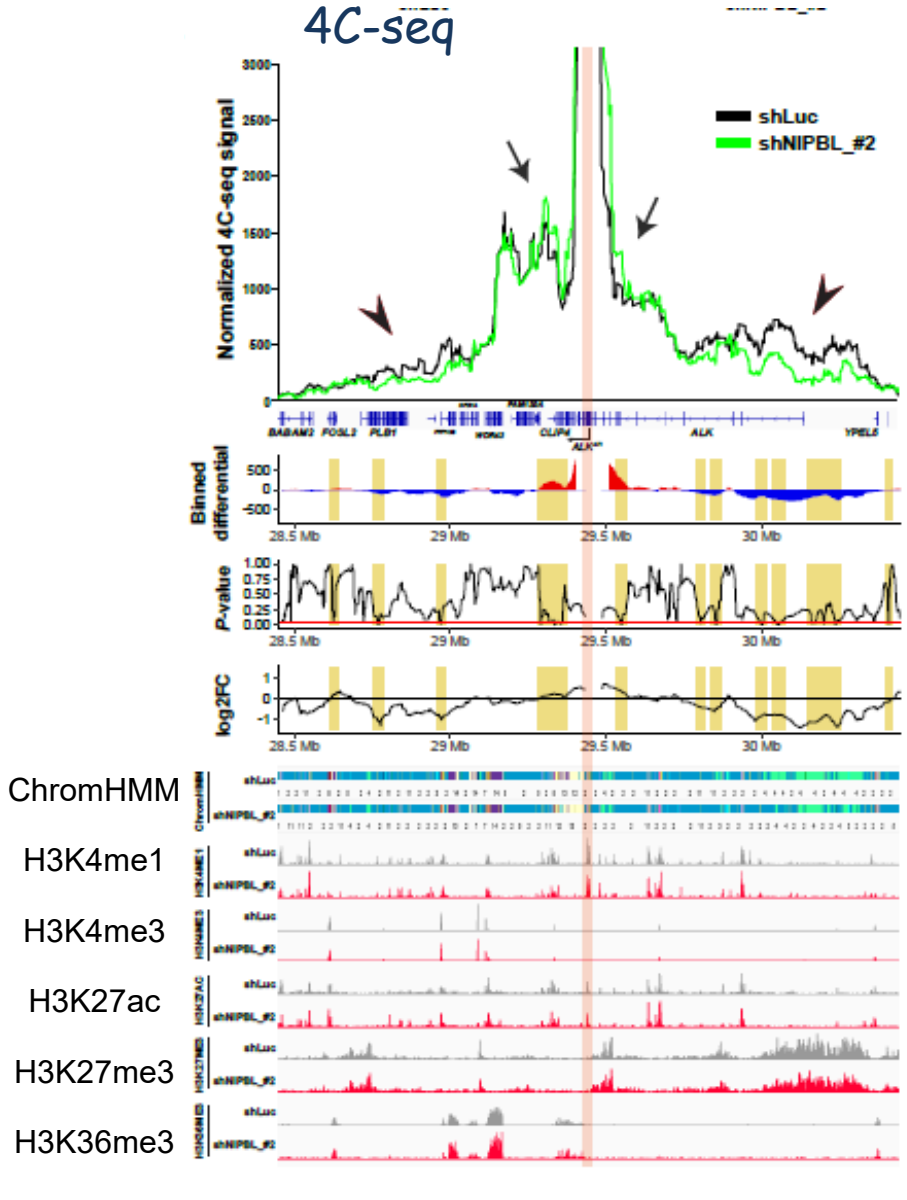
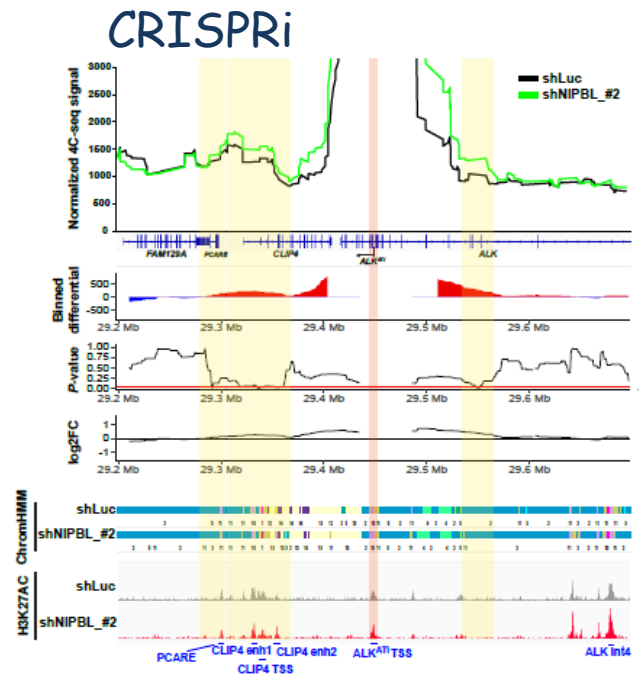
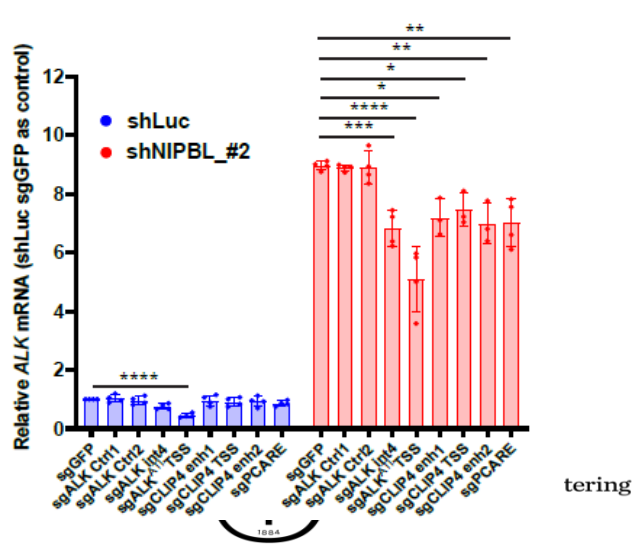
TAD hierarchy loss results in alternative promoter activation from E-altP retargeting (switch from long-range E to short-intermediate range E)









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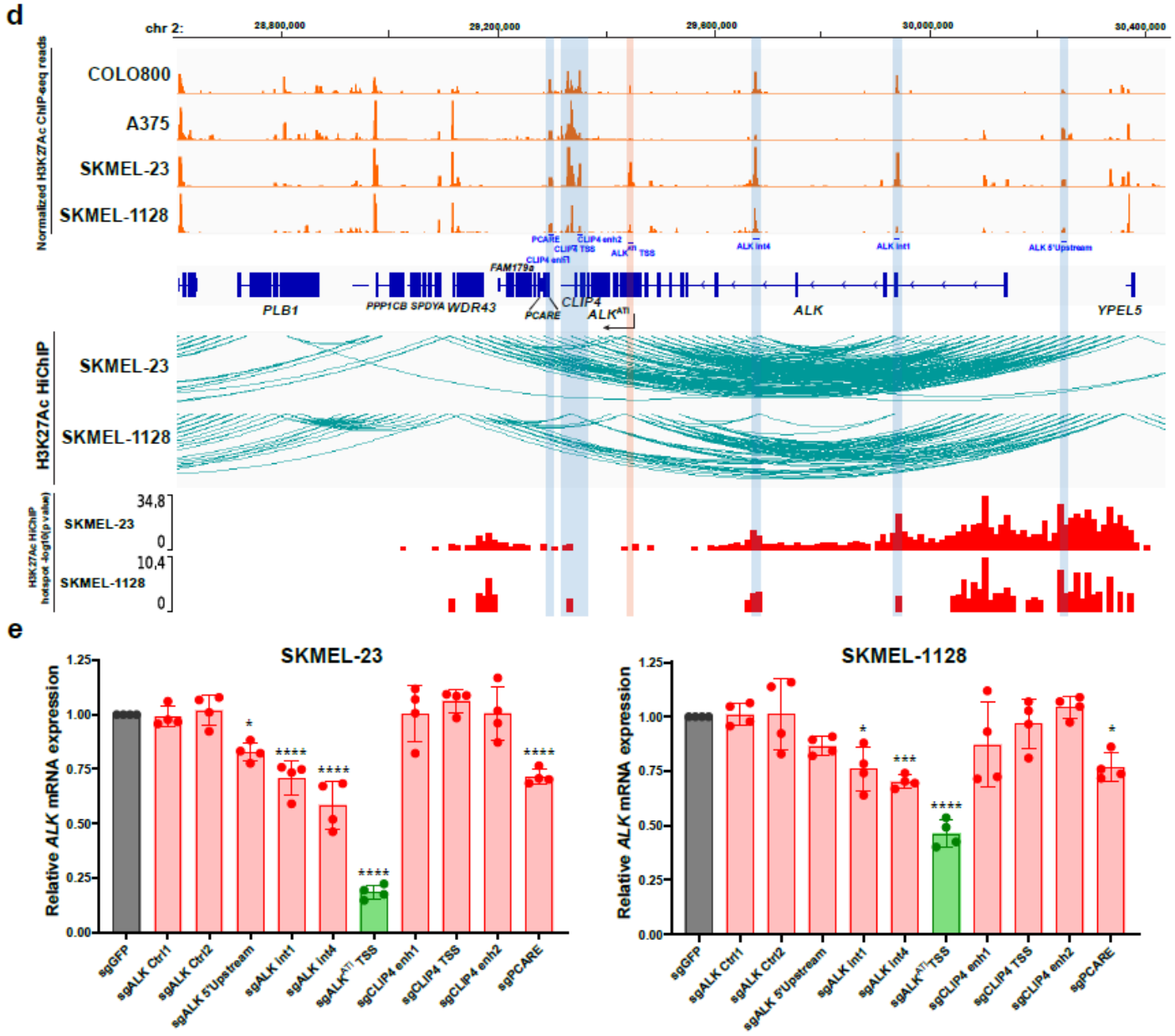
NIPBL perturbation contributes to transcriptional activation of *ALK*^{ATI} from the alternative promoter through enhancer re-targeting in melanoma



ALK^{ATI} expression is dependent on MITF

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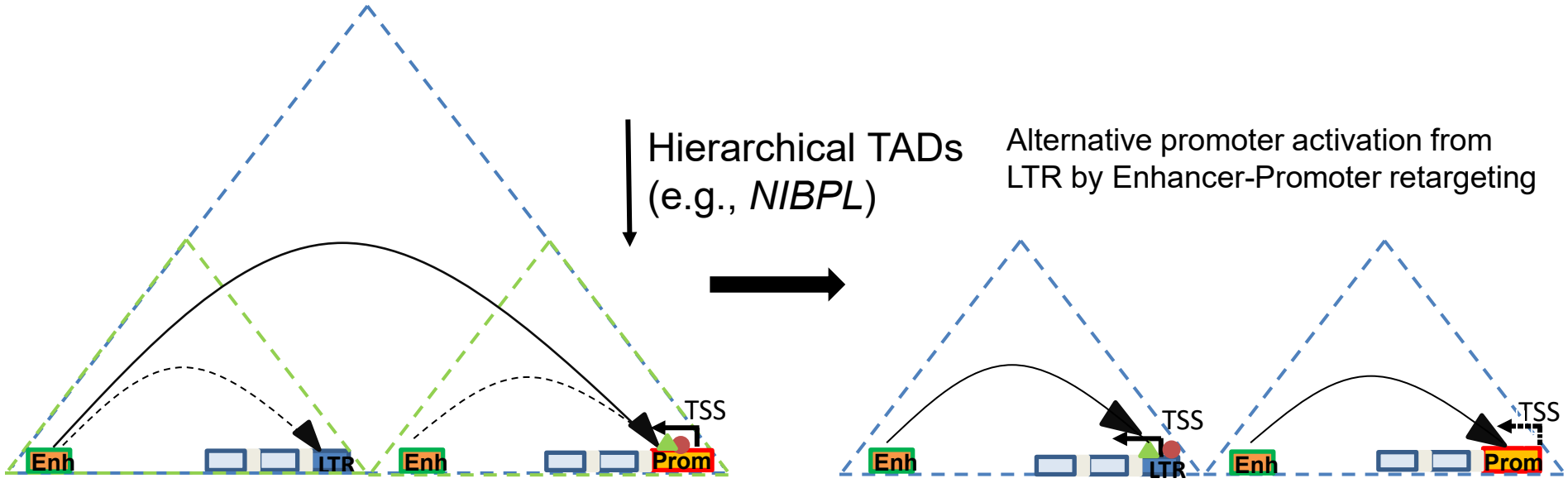
Alternative promoter usage in cancer via perturbation in 3D chromatin hierarchy



Hierarchical 3D chromatin organization

Physiologically – Hierarchical TAD organization suppresses LTR-mediated spurious transcription and regulate LTR co-option, particularly in active chromatin compartment.

Pathologically – Perturbation in hierarchical TAD organization can lead to oncogene activation, transcriptional diversity/plasticity, neoepitope expression.

Paper discussion…

Discussion Paper: https://pubmed.ncbi.nlm.nih.gov/31666694/

Flavahan WA et al., Altered chromosomal topology drives oncogenic programs in SDH-deficient GISTs. Nature 2019

Published: 23 December 2015

Insulator dysfunction and oncogene activation in *IDH* mutant gliomas

William A. Flavahan, Yotam Drier, Brian B. Liau, Shawn M. Gillespie, Andrew S. Venteicher, Anat O. Stemmer-Rachamimov, Mario L. Suvà & Bradley E. Bernstein